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MORPHOLOGIC CHANGES IN THE HUMAN KIDNEY FOLLOWING PROLONGED ADMINISTRATION OF ALKALI

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In the clinical studies of alkalosis previously reported [1] evidence was presented indicating that although the urea clearance frequently diminished during alkalosis, renal function invariably returned to its original level when the acid-base balance was restored. It was further shown that prolonged ingestion of alkali likewise did not permanently lower renal function. On the basis of these observations it did not appear likely that either alkalosis or prolonged alkali therapy produced irreversible anatomic renal injury. This view was supported by the absence of significant anatomic changes in the kidneys of the dog after long-continued administration of large quantities of sodium bicarbonate.[2] To secure additional information on this problem, we undertook a histologic study of the kidneys of patients who had received large amounts of alkali and who had died either in alkalosis or of other complications.

Few studies have appeared in the literature describing the effects of alkali therapy and of alkalosis complicating antacid treatment on the structure of the human kidney.

Hardt and Rivers [3] reported the case of a 66 year old man who had taken sodium bicarbonate intermittently for fifteen years for distress caused by ulcer. After he had been in the hospital under intensive alkali therapy for fifteen days, he became stuporous and subsequently died despite the use of saline infusions. The plasma carbon dioxide had measured 44.9 and 50.4 millimols per liter, and the

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1. Kirsner, J. B., and Palmer, W. L.: J. A. M. A. **116**:384, 1941; Arch. Int. Med. **69**:789, 1942.

2. Kirsner, J. B.: Arch. Path. **32**:76, 1941.

3. Hardt, L. L., and Rivers, A. B.: Arch. Int. Med. **31**:171, 1923.

blood urea nitrogen 134 and 212 mg. and the blood creatinine 4.3 mg. per hundred cubic centimeters. Necropsy revealed a duodenal ulcer, bilateral bronchopneumonia and generalized arteriosclerosis. Microscopic examination of the kidneys disclosed focal and diffuse interstitial lymphocytic infiltration, sclerosis of some glomeruli and complete hyalinization of others, thickening of most of Bowman's capsules, and sclerosis of the arteries and arterioles.

Cooke [4] reported the case of a 46 year old man who had taken alkalis for one year. The patient had been vomiting for five weeks and was admitted to the hospital in a semicomatose condition. After the administration of saline solution the serum carbon dioxide decreased from 56.2 to 31.8 millimols per liter and the plasma sodium chloride rose from 353 to 546 mg. per hundred cubic centimeters. The blood urea nitrogen increased, however, from 165 to 252 mg. per hundred cubic centimeters, and the patient died within several days. The kidneys were large, smooth and engorged with blood. The microscopic changes were interpreted as indicative of "chronic interstitial nephritis" with terminal acute nephritis.

Oakley [5] described the anatomic findings in 2 patients who died in alkalosis after alkali therapy: 1. A 63 year old man with a duodenal ulcer had been treated with a mixture of magnesium carbonate, sodium bicarbonate and bismuth oxycarbonate. The patient had received 90 Gm. of sodium bicarbonate in four days, at the end of which time he became irrational and comatose; he died four days later. The blood urea nitrogen had measured 140 mg. per hundred cubic centimeters. The kidneys were normal grossly, and the only microscopic changes recorded were swelling and focal fatty degeneration of the tubular epithelium. 2. A 64 year old man, treated similarly, had received 541 Gm. of sodium bicarbonate in eleven days. Symptoms of alkalosis appeared on the eleventh day, and the patient died three days later. The blood urea nitrogen twenty-four hours before death was 250 mg. per hundred cubic centimeters; the urine was reported as normal. Microscopic study of the kidneys revealed only a slight amount of fibrosis, occasional arteriosclerotic changes and a few small calcareous deposits.

METHOD OF STUDY

The present investigation comprises a study of the autopsy observations in 14 patients who had received during life large quantities of alkali and for whom adequate studies of acid-base balance were available. The series is composed of (group 1)) 4 patients who had taken large amounts of alkali but in whom alkalosis had never occurred; (group 2) 6 patients who had experienced one or more episodes of alkalosis during alkali therapy but who did not die in alkalosis (5 of this group died of other complications several weeks after alkalosis, while the sixth patient died ten months after severe alkalosis), and (group 3) 4 patients who had taken large quantities of alkali and in whom alkalosis was present at the time of death.

RESULTS

Group 1. Prolonged Alkali Therapy—No Alkalosis.

CASE 1.— A 52 year old man had been treated for symptoms of ulcer for seven years, during which time he had received large amounts of alkali. He entered the hospital because of a severe hemorrhage, which proved fatal on the twenty-

4. Cooke, A. M.: Quart. J. Med. **1**:527, 1932.

5. Oakley, W. M.: Lancet **2**:187, 1935.

ninth day despite six blood transfusions and the subcutaneous administration of a 5 per cent solution of dextrose and a physiologic solution of sodium chloride. Five hundred and eighty-five grams of sodium bicarbonate and calcium carbonate was administered during the first fifteen days; the patient received no alkali during the last fourteen days. The acid-base balance was normal. There was no elevation of blood urea or of nonprotein nitrogen, and the urea clearance was 69 per cent of average normal. The urine contained hyaline and granular casts.

Necropsy revealed the following conditions: a benign peptic ulcer of the lesser curvature of the stomach with recent thrombosis of the artery in the floor of the ulcer; acute distention of the right side of the heart with acute generalized passive hyperemia, most evident in the kidneys; pulmonary edema; right hydrothorax; ascites; moderate sclerosis of the aorta and coronary arteries.

The kidneys were purplish and firm; the right weighed 185 Gm. and the left 160 Gm. The capsule of each stripped easily, leaving a smooth surface. The parenchyma was cyanotic. Microscopic examination revealed only mild arteriosclerotic changes.

CASE 2.—A 56 year old man had suffered from high blood pressure for at least four years. One year, five months, and one month previous to his admission to the hospital he had experienced hemorrhages arising from a gastric ulcer. Alkali had been taken intermittently during this time. He entered the hospital with another massive hemorrhage. The therapy included two blood transfusions and 1,521 Gm. of sodium bicarbonate and calcium carbonate given over a period of thirty-nine days. The acid-base balance was normal. The blood urea nitrogen was 9.6 mg. per hundred cubic centimeters and the urea clearance 89 per cent of average normal; nine examinations of the urine gave negative results. The patient returned two months later because of a recurrence of the gastric ulcer, hypertensive vascular disease and hypertensive retinitis. Alkali had been taken occasionally. The blood pressure then was 188 systolic and 120 diastolic. Three hundred and ninety-three grams of sodium bicarbonate and calcium carbonate was administered in nine days. The acid-base balance remained normal; the blood urea nitrogen measured 14 mg. per hundred cubic centimeters. Six specimens of urine were normal. One year after the initial entry the patient returned for a third time. He received, over a period of fifty-nine days, 2,301 Gm. of sodium bicarbonate and calcium carbonate. The blood urea nitrogen amounted to 12.3 mg. per hundred cubic centimeters, and the urea clearance was 94 per cent of average normal. The lesion of the stomach discovered at this time was diagnosed as carcinoma. The patient died several months later.

Necropsy revealed diffuse carcinomatous infiltration of the stomach with pyloric obstruction. There was a healed benign gastric ulcer in the prepyloric region, as well as a chronic benign ulcer high on the lesser curvature, with fixation to the tail of the pancreas. Other conditions were: bronchopneumonia of the right upper pulmonary lobe and an early abscess in the right lower lobe, bilateral hydrothorax and arteriosclerosis of the aorta.

Each kidney weighed 140 Gm. and was flabby in consistency. The capsule stripped readily, leaving a uniformly granular pale purple-gray surface. A few small cysts were present on the surface. The cortex measured 3 to 4 mm.; the corticomedullary demarcation and the cortical markings were obscured. Several small cysts were located in the medulla. The renal pelves and ureters were normal. Microscopic examination revealed a few areas of scarring with foci of round cells. Some of the glomeruli showed varying stages of hyalinization; the associated tubules were atrophied. The tubules, especially those in the cortex, showed

moderate fatty changes and contained protein debris and hyaline casts. There was some increase in interstitial fibrous tissue. The arteries and arterioles showed varying degrees of sclerosis.

CASE 3.—A 62 year old man had experienced gastrointestinal distress for ten years. Alkali had been taken regularly for about one year and then intermittently during the subsequent nine years. He entered the hospital because of a severe hemorrhage, and for the initial five days was treated with alkali, receiving 320 Gm. of calcium carbonate. Six blood transfusions were given. Subtotal gastrectomy was performed because of persistent massive hemorrhage. The patient continued to bleed, however, and he died shortly after the operation. There was no alkalosis.

Necropsy revealed the following conditions: a large chronic benign ulcer on the posterior wall of the stomach near the cardiac portion and close to the greater curvature; perforation of an aneurysm of a short gastric artery located in the floor of the ulcer; marked parenchymatous changes in the myocardium with moderate dilatation; focal necrosis of the liver; marked bilateral hypostatic pulmonary edema with aspirated blood and probably beginning bronchopneumonia.

Each kidney weighed 130 Gm.; the surrounding fat and capsule stripped easily. The capsular surfaces were smooth except for a few tiny retention cysts. The vascular markings were fairly well preserved. The renal pelves were normal. The chief microscopic observations were scattered hyalinized glomeruli and slight fibrous thickening of the walls of the larger arteries. Occasional cortical tubules showed fatty degeneration.

CASE 4.—A 57 year old man had experienced gastrointestinal distress for fifteen years. One year prior to entry he had been treated with moderate amounts of alkali following the appearance of tarry stools. Sodium bicarbonate had been taken occasionally for three weeks prior to entry. The patient received a total of 1,212 Gm. of sodium bicarbonate and calcium carbonate over a period of twenty-three days. He died on the twenty-fourth day as a result of coronary thrombosis. The acid-base balance was normal. The blood urea nitrogen was 14.8 mg. per hundred cubic centimeters and the urea clearance 78 per cent of the average normal. One of ten specimens of urine contained albumin.

Necropsy revealed the following conditions: multiple healed infarcts of the myocardium; marked coronary sclerosis with recent rupture of a plaque; peptic ulcer of the first part of the duodenum; cardiac hypertrophy and dilatation; congestion of the liver and spleen; nodose goiter with solitary cyst.

The kidneys each weighed 240 Gm. and were very firm. The capsules stripped easily, leaving smooth, pale red surfaces with a few cysts, measuring up to 5 mm., and small irregular stellate depressed scars. The cut surfaces appeared cyanotic. The cortex of each kidney was 7 mm. wide; the corticomedullary demarcation was distinct, although the other markings were not clear. Microscopic examination revealed scattered hyalinized glomeruli and focal round cell infiltration. Protein debris was present in the lumens of the tubules. The epithelium of the tubules contained small amounts of fat. There were moderate sclerosis of the arteries and marked dilatation and congestion of the capillaries.

No significant renal changes were noted in these 4 cases despite the prolonged use of considerable quantities of alkali. The hyalinized glomeruli and atrophied tubules observed in cases 2 and 4 are usual autopsy findings at this age. The focal fatty degeneration of the tubular

epithelium and the protein precipitate and hyaline casts observed in 2 cases are acute changes attributable to the passive congestion and anemia.

Group 2. Prolonged Alkali Therapy with Alkalosis—Death Caused by Other Complications.

CASE 1.—A 60 year old man had experienced distress from a peptic ulcer for four years. He occasionally took sodium bicarbonate and frequently induced vomiting to obtain relief from pain. He entered the hospital because of a severe hemorrhage and died sixteen days later despite eight blood transfusions. Six hundred and ninety-five grams of sodium bicarbonate, calcium carbonate and tricalsate[6] was administered during this period. Studies of the acid-base balance revealed moderate alkalosis on the third day (carbon dioxide 36.6 millimols per liter and p_H 7.53); this became severe on the seventh day with the serum carbon dioxide measuring 46.3 millimols per liter and the p_H 7.63. The alkalosis subsided rapidly after the discontinuance of the alkali therapy and with the administration of ammonium and sodium chloride by mouth. Alkali therapy (tricalsate) was resumed on the tenth day, but the acid-base balance remained normal. The blood urea nitrogen, which had risen to 46.8 mg. per hundred cubic centimeters on the eighth day, decreased to 19.4 mg. one week later. The urea clearance values ranged from 40 to 79 per cent of average normal. The blood creatinine fluctuated between 1.29 and 1.88 mg. Seven examinations of the urine gave negative results.

Necropsy revealed a massive hemorrhage into the stomach and duodenum from a branch of the superior pancreaticoduodenal artery located in the floor of a large duodenal ulcer; the lungs and retroperitoneal tissues were markedly edematous and both pleural cavities contained fluid.

The kidneys were embedded in considerable adipose tissue; together they weighed 320 Gm. The capsule of each stripped easily, leaving a smooth surface. The cortices were extremely pale and measured 5 to 6 mm.; the medullae measured 15 mm. The cortical markings were fairly distinct. The renal pelves were normal. Microscopic examination revealed most of the glomeruli to be normal; fat stains demonstrated a few foci of slight fatty degeneration in the tubules. There was moderate intimal thickening of the larger arteries with extreme thickening of some arterioles.

CASE 2.—A 54 year old man had experienced distress from ulcer for eighteen years, for which sodium bicarbonate had been taken frequently. He was hospitalized for twenty-one days and during the first nine days received 137 Gm. of sodium bicarbonate and calcium carbonate. Symptoms of alkalosis appeared on the ninth day. The serum carbon dioxide at this time was 46.5 millimols per liter and the p_H 7.58. The blood urea nitrogen increased from 9.2 mg. to 18.1 mg. per hundred cubic centimeters and the urea clearance, previously 63 per cent of average normal, decreased to 37 per cent. Alkalis were stopped for six days, and sodium and ammonium chloride were given by mouth. The acid-base balance returned to normal in six days; the blood urea nitrogen decreased to 10.9 mg. per hundred cubic centimeters, and the urea clearance increased to 65 per cent. Tricalsate then was substituted for the previous alkali, and the patient's subse-

6. Tricalsate is a preparation which when in aqueous suspension consists essentially of tribasic calcium phosphate and sodium citrate.

quent clinical course was uneventful. He died ten months later, however, after an operation for carcinoma of the gallbladder.

Necropsy revealed carcinoma of the gallbladder with metastases to the liver and perihepatic lymph nodes, hemorrhage into the duodenum from a duodenal ulcer and bilateral pulmonary edema.

The kidneys were of about equal size and together weighed 300 Gm. The capsules stripped easily leaving smooth surfaces. The cortices were slightly brown; the cortical markings were preserved. Microscopically the glomeruli were normal. There was no significant change in the tubular epithelium. The only microscopic change was slight diffuse subcapsular scarring with round cell infiltration.

CASE 3.—A 38 year old man had had a stenosing duodenal ulcer for fifteen years complicated by a massive hemorrhage eight years previously. He had vomited frequently since that time and often had required sodium bicarbonate for the relief of his distress. The blood pressure was found to be 190 systolic and 130 diastolic. Alkali, mainly sodium bicarbonate, was given to the amount of 1,292 Gm. during the first seventeen days. Partial gastrectomy was performed on the eighteenth day because of obstruction. The patient vomited persistently and died several days later despite the parenteral administration of large quantities of physiologic solution of sodium chloride and 5 per cent dextrose solution. The serum carbon dioxide and p_H were elevated slightly on the fiftieth hospital day; the blood urea nitrogen was 25.5 mg. per hundred cubic centimeters and the urea clearance 39 per cent of average normal. Two of six specimens of urine contained hyaline and granular casts, albumin and occasional red and white blood cells.

Necropsy revealed the following conditions: a chronic duodenal ulcer with adherence to the pancreas; dilatation of the duodenum; torsion and gangrene of the proximal anastomosed jejunal loop with dilatation, hemorrhage and venous thrombosis; hemoperitoneum; cardiac hypertrophy.

The kidneys each weighed 160 Gm.; they were markedly pale, gray-white and firm. The capsules stripped easily, leaving smooth surfaces. The cortices measured 5 to 6 mm.; the cortical markings were distinct. On microscopic examination, many of the glomeruli showed varying degrees of hyalinization, and some were partially or completely adherent to the capsules. There was marked thickening of Bowman's capsules. The epithelium of some of the collecting tubules showed mild degenerative and regenerative changes,[7] and the lumens of these tubules contained blue-staining masses (with the hematoxylin and eosin stain) suggesting calcium. Protein precipitate was noted occasionally. There was a definite increase in the interstitial fibrous tissue; scattered foci of round cells were present. Both the large and the small renal arteries showed intimal proliferation.

CASE 4.—A 34 year old man had experienced symptoms of ulcer for five years, during which time he had taken moderate amounts of sodium bicarbonate. Hypertension had been present for at least six years. He entered the hospital with a severe hemorrhage and received, over a period of forty-nine days, 2,700 Gm. of alkali, consisting of sodium bicarbonate, calcium carbonate and tricalsate. Studies

7. Regenerative changes accompanying degeneration of the epithelium of the renal tubules are frequently observed. Experimentally, they have been noted to occur within a period of seven days.

of the acid-base balance revealed mild alkalosis. Renal function was impaired, the urea clearance values ranging from 26 to 47 per cent of average normal. The blood urea nitrogen rose to 43.8 mg. per hundred cubic centimeters but gradually decreased by the forty-fourth day to 12.3 mg. After recovery from the hemorrhage the patient was continued on a modified ulcer regimen with tricalsate for five months. At this time he experienced a second severe hemorrhage, from which he died on the thirty-second day. Twenty-four hours before death the patient experienced a transfusion reaction characterized by a chill and a rise of temperature to 106 F. Treatment had included the administration of small amounts of sodium bicarbonate, subcutaneous injection of physiologic solution of sodium chloride and 5 per cent dextrose solution, and nine blood transfusions. There was no evidence of alkalosis at this time. The blood urea nitrogen, however, ranged from 40.2 to 113.2 mg. per hundred cubic centimeters and the urea clearance from 9 to 24 per cent of average normal. The blood creatinine varied from 2 to 3 mg. per hundred cubic centimeters. Five of nine specimens of urine contained a trace of albumin; the maximum specific gravity was 1.018.

Necropsy revealed the following conditions: an acute duodenal ulcer with evidence of a recent massive hemorrhage; a chronic healed duodenal ulcer; marked cardiac hypertrophy, especially of the left ventricle; marked pulmonary edema with early bronchopneumonia; parenchymatous degeneration of the liver and of the myocardium; moderate sclerosis of the aorta and of the coronary and medium-sized arteries.

The right kidney weighed 90 Gm. and the left 110 Gm.; they were flabby and pale. The capsule of each stripped easily, leaving a finely granular surface with numerous punctate hemorrhages. The cortices were somewhat decreased in width, and the cortical markings were indistinct. The renal pelves were normal. Microscopic examination revealed a marked increase in fibrous tissue with diffuse and focal round cell infiltration. Many of the glomeruli showed varying degrees of hyalinization. Many of Bowman's capsules were thickened. These changes were associated, especially in the cortices, with marked dilatation of tubules in groups, the epithelium of which was flat and cuboidal. Hyaline casts were noted frequently. No calcium precipitate was visible. Fat stains disclosed focal fatty degeneration of the tubular epithelium. The arteries and arterioles were markedly sclerosed, and the walls of the arterioles showed severe fatty degeneration.

Case 5.—A 57 year old man entered the hospital because of massive hemorrhage from a gastric ulcer. No alkali had been taken previously. He remained in the hospital for sixty-six days, during which time he received a total of 2,700 Gm. of sodium bicarbonate and calcium carbonate. Nine examinations of the urine gave negative results. The serum carbon dioxide amounted to 33.6 millimols per liter and the p_H was 7.58 on the seventh hospital day. He returned three and one-half years later with a recurrence of the ulcer; alkalis had been taken during the interim. The patient received 2,574 Gm. of sodium bicarbonate and calcium carbonate over a period of sixty-six days. Studies of the acid-base balance on the third, eleventh and twenty-eighth days revealed mild to moderately severe alkalosis, the serum carbon dioxide ranging from 32.4 to 37.8 millimols per liter, the p_H from 7.51 to 7.63 and the chloride from 90.6 to 98.2 millimols per liter. The blood urea nitrogen varied from 15.8 to 20.8 mg. per hundred cubic centimeters and the urea clearance from 41 to 109 per cent of the average normal. In eleven examinations the urine was normal. Six months later (four years after

the initial entry) the patient was hospitalized because of a recurrence of hemorrhage; alkalis had been taken occasionally during this interval. He remained in the hospital for two hundred and eleven days, receiving a total of 6,933 Gm. of alkali (mainly calcium carbonate), which was almost entirely administered during the first one hundred and seventy-three days. Aspiration of the gastric contents yielded from 300 to 400 cc. nightly. Many determinations of the acid-base balance and of renal function (urea clearance) were made, as shown in table 1.

Twenty-seven examinations of the urine gave negative results; the maximum specific gravity was 1.030. During the last eighty days in the hospital the patient's clinical course was complicated by the development of auricular flutter. He also experienced severe bouts of coughing. Cyanosis, which had been present to a mild degree, increased. The treatment included parenteral administration of a 5 per cent dextrose solution and physiologic solution of sodium chloride and blood

TABLE 1.—*Acid-Base Balance and Urea Clearance in Case 5*

Hospital Day	Serum Cl, mM per Liter	Serum CO_2, mM per Liter	pH	Blood Urea N, Mg. per 100 Cc.	Urea Clearance, % Av. Normal
120th	100.2	39.0	7.53	15.1	79
137th	92.1	36.1	7.52	14.9	91
141st	90.5	35.3	7.46		
152d	84.3	45.8	7.53		
153d	....	43.2	7.48	19.7	
162d	88.6	32.6	7.47	16.1	61
164th	100.8				
168th	88.4	33.4	7.47	40.4	
172d	93.8	30.4	7.43		
179th	94.1	33.5	6.50	9.2	87
183d	91.4	33.3	7.50	10.1	63
186th	....	34.0	7.50	9.1	80
190th	....	36.2	7.57	14.6	
193d	88.0	37.4	7.53	17.4	102
200th	89.0	35.0	7.48	25.0	73
207th	95.0	26.4	7.41	43.9	41
210th	92.3	23.4	7.33	63.5	
211th	Died				

transfusions. The patient, however, did not improve, and he was found dead in bed on the two hundred and eleventh hospital day.

Necropsy revealed the following conditions: marked atrophic pulmonary emphysema; senile pulmonary bullous emphysema with marked hypertrophy of the myocardium on the right (cor pulmonale); acute pulmonary edema; acute distention of the right ventricle with acute generalized passive congestion; "nutmeg" liver; chronic induration of the spleen; bilateral hydrothorax; massive benign peptic ulcer of the lesser curvature in the prepyloric area of the stomach; chronic atrophic gastritis; old perforative peptic ulcer with adhesions to the liver; moderate sclerosis of the pulmonary artery and of the aorta.

The right kidney weighed 180 Gm.; the left, 155 Gm. The cortical markings were somewhat obscured. The capsules stripped readily from smooth pale surfaces. There appeared to be an increased amount of yellowish white material in the cortices and pyramids. The renal pelves and ureters were normal. On microscopic examination, most of the glomeruli were normal. A few were hyalinized, and the corresponding tubules were atrophied. Considerable protein precipitate

was present in Bowman's spaces. The epithelium of the convoluted tubules was markedly swollen, so that the lumens were almost obliterated in occasional areas. Some of the nuclei were hyperchromatic, while others had disappeared. Occasional hyaline casts were visible in the collecting tubules. A few of the collecting tubules contained blue-staining material suggesting calcium; small foci of round cells occasionally surrounded these tubules. There was marked fatty degeneration, particularly of the collecting tubules, although some of the convoluted tubules also were affected.

CASE 6.—A 52 year old man had experienced gastrointestinal distress for three years, during which time he had had four hemorrhages. Large amounts of alkali were taken by him. The treatment included administration of 400 Gm. of calcium carbonate and magnesium oxide, given during the first twenty-two days, and aspiration of the stomach daily for the same length of time, the aspirates averaging 300 cc. in amount. On the twenty-third hospital day cholecystectomy and

TABLE 2.—*Acid-Base Balance and Urea Clearance in Case 6*

Hospital Day	Serum Cl, mM per Liter	Serum CO_2, mM per Liter	p_H	Blood Urea N, Mg. per 100 Cc.	Urea Clearance, % Av. Normal	Non-protein Nitrogen, Mg. per 100 Cc.	Creatinine, Mg. per 100 Cc.	Total Base, mEq. per Liter
3d	94.0	30.7	....	....	..	53.6	....	.....
4th	....	....	....	40.7	15	....	....	.....
10th	72.0	45.0	7.62	65.1	..	....	3.37	.....
14th	86.0	38.1	7.63	45.2	10	....	....	.....
18th	97.8	32.9	7.52	43.5	22	....	....	.....
21st	....	30.0	7.53	38.8	16	....	....	.....
23d	Posterior gastroenterostomy and cholecystectomy							
48th	98.8	24.9	7.47	17.8	22	....	....	.....
60th	98.0	26.4	7.34	20.4	37	....	....	.....
66th	97.6	22.8	7.32	27.3	33	....	....	138.6
73d	98.2	20.4	7.38	26.6	30	....	....	.....
75th	....	16.7	....	....	..	....	....	.....
77th	96.3	16.8	....	....	..	....	....	140.6
79th	....	10.1	....	....	..	42.5	....	Died

posterior gastroenterostomy were performed for cholelithiasis and an obstructing duodenal ulcer. No alkali was given postoperatively. The patient's course was complicated by infection of the wound, which was treated with sulfanilamide. The application of sulfanilamide was discontinued after four days, however, because of the toxic reaction. Large quantities of physiologic solution of sodium chloride, 5 per cent saline solution, Ringer's solution and 5 per cent dextrose solution were given parenterally. Wangensteen suction of the stomach was carried out for fourteen days postoperatively, the quantities removed varying from 500 to 3,000 cc. in amount daily. A number of specimens of urine contained albumin. Determinations of acid-base balance and renal function (urea clearance) were made frequently, as shown in table 2.

The patient's condition gradually became worse, and he died on the fifty-sixth postoperative day of bronchopneumonia.

Necropsy revealed generalized latent osteitis fibrosa cystica, hyperplasia of the parathyroid glands, pyloric stenosis, bronchopneumonia of the right lower pulmonary lobe and generalized arteriosclerosis.

The kidneys were small and pale; together they weighed 165 Gm. The capsules stripped easily from smooth surfaces. The cortical markings were obscured. The pelvic fat was increased, and each renal pelvis was dilated and thick walled.

The calices were large and clubbed on the ends. The ureters were normal. Microscopic study revealed distinctly abnormal kidneys. The tips of the pyramids were not recognizable. In the cortices many wedge-shaped areas of scarring and chronic inflammation alternated with more normal areas. The ratio of scarred to normal tissue was estimated to be 1:2. Remnants of hyalinized glomeruli and some partially hyalinized and shrunken tubules were visible in the scarred areas. The inflammatory cells of the scars consisted mostly of small mononuclears and lymphocytes. Most of the glomeruli in the more normal areas were well preserved. The tubular epithelium in the well preserved regions varied from flat cuboidal to tall cells; the nuclei varied greatly in size. Many of the tall cells contained pale, granular or vacuolated cytoplasm, and some contained colloid droplets. Casts were infrequent, and there was little protein debris. Numerous cast-filled and cast-obstructed collecting tubules were seen. Most of the casts were composed of deep blue or blue-purple staining granular masses forming dense aggregates, suggesting calcium. Similar masses were present in the interstitial tissue with no recognizable tubular membrane surrounding them. The presence of these was associated with an increase in fibrous tissue but with little inflammatory reaction. The larger arteries were moderately hyalinized but not greatly narrowed. The small arteries, especially those in the scarred regions, were thickened.

No significant renal changes were noted in cases 1 and 2 despite the previous existence of severe alkalosis. The scattered hyalinization of glomeruli and the focal fatty degeneration in the tubular epithelium are usual necropsy findings in patients of this age.

The alkalosis in cases 3 and 4 had been mild. The hypertension which had been present in both patients undoubtedly accounted for all the changes described. An additional observation in case 3 was the presence in a few collecting tubules of blue-staining material suggestive of calcium; this finding is discussed later.

Case 5 constitutes one of the most interesting of the entire series. The patient had consumed enormous quantities of alkali, receiving during three hospital periods alone 12,207 Gm. of sodium bicarbonate and calcium carbonate.. Large amounts of the gastric contents had been aspirated daily, and the patient had experienced one episode each of mild, moderate and severe alkalosis. There was little anatomic evidence of chronic renal injury despite these factors. Most of the glomeruli were normal. The swelling of the tubular epithelium with focal necrosis and fatty degeneration and the presence of protein precipitate and hyaline casts are attributable to the marked passive congestion. Blue-staining material was visible in occasional collecting tubules in this case also.

Case 6 is likewise of considerable interest. The aspiration of large quantities of the gastric contents undoubtedly had contributed to the development of the severe alkalosis. It should be emphasized that this patient exhibited clinical evidence of renal impairment at the onset of therapy. Many of the renal changes were arteriosclerotic in origin.

Most of the glomeruli and approximately two thirds of the entire substance of each kidney appeared normal, however. The most striking observation was that of blue-staining material in numerous collecting tubules, some of which were obstructed and surrounded by an increased amount of interstitial fibrous tissue. Similar blue-staining material was visible also in the interstitial tissue itself, suggesting possibly a primary type of calcification in contrast to calcium precipitation. The parathyroid hyperplasia presumably was secondary to the renal insufficiency. The role of sulfanilamide toxicity in the genesis of the renal changes cannot be evaluated but, on the other hand, cannot be completely excluded.

Group 3. Prolonged Ingestion of Massive Quantities of Alkali, with Death in the Course of Alkalosis.

CASE 1.—A 59 year old man had for three years frequently required sodium bicarbonate for the relief of symptoms of ulcer. He was hospitalized for fourteen days, during which time he received a total of 609 Gm. of sodium bicarbonate, calcium carbonate and tricalsate. Moderately severe chemical alkalosis was noted on the fifth and ninth days. One year and seven months later the patient's symptoms recurred, and he was instructed to take alkalis at hourly intervals and to aspirate his stomach each night. This program was continued for two months; the gastric content aspirated varied in amount from 3 to 6 ounces (93 to 186.5 cc.). Several days before hospitalization he began to vomit large amounts of gastric content, and just prior to entry he became disoriented and semicomatose. He was markedly dehydrated and emaciated; the blood pressure which formerly had been 140 systolic and 85 diastolic was now 80 systolic and 52 diastolic. Massive quantities of physiologic solution of sodium chloride, 5 per cent saline solution and 5 per cent dextrose solution were administered subcutaneously and intravenously. Two blood transfusions also were given. The patient failed to respond to this therapy; he became markedly cyanotic and died on the fifth hospital day. The serum carbon dioxide on the third day measured 42.6 millimols per liter; this diminished to 33 millimols twenty-four hours later. The blood chloride increased from 494 to 653 mg. per hundred cubic centimeters, while the nonprotein nitrogen varied between 140 and 146 mg. per hundred cubic centimeters.

Necropsy revealed the following conditions: a chronic duodenal ulcer near the pylorus; marked duodenal stenosis, exaggerated by kinking due to traction of the stomach on the hepatoduodenal ligament; gastric hypertrophy and dilatation; slight cardiac dilatation; marked bilateral hypostatic pulmonary hyperemia and edema; acute aspiration bronchopneumonia with beginning suppuration in the lower lobe of the right lung; fatty degeneration and hemosiderosis of the liver; extreme edema of the entire gastrointestinal tract; ascites; minimal senile arteriosclerosis.

The kidneys were dark purple-gray and firm; the perirenal fat was slightly adherent; the right kidney weighed 100 Gm. and the left 120 Gm. The capsules stripped easily, leaving very cyanotic surfaces with prominent stellate veins. The cortices were dark reddish purple; the cortical rays were indistinct; the cortices measured 6 mm. The corticomedullary area was very dark; the tips of the pyramids were quite pale and fibrous. Microscopic examination revealed a few foci of scarring under the capsules with occasional round cell infiltration. There was marked increase in interstitial fibrous tissue in the medullary pyra-

mids. Occasional scattered glomeruli were hyalinized, but the majority were practically normal. Bowman's spaces were well filled and nowhere were greatly dilated. Many of the tubules in the cortices had wide lumens and flat epithelium, and usually contained protein debris. A few tubules in the scarred areas of the kidneys were atrophic. Throughout the cortices single or several tubules contained epithelium with large pleomorphic nuclei. Occasional tubules contained pale to dark blue-staining granular masses. A few of these masses appeared to be obstructing the tubules, the epithelium of which was flat or focally missing. The obstructed tubules were located usually not far from the corticomedullary junction; none were recognized in Henle's loops. There was no marked sclerosis of the large or of the small arteries. Sudan IV stains demonstrated slight fatty degeneration of the tubular epithelium.

Case 2.—A 49 year old man had experienced symptoms of ulcer for seven years. Large amounts of alkali had been taken during this time. His right kidney had been removed six years previously because of a tuberculous infection. Just prior to hospitalization the patient had taken huge amounts of sodium bicarbonate and had vomited frequently. Forty-two grams of calcium carbonate was given during the first six days. The patient continued to vomit, and on the seventh day the serum carbon dioxide measured 57.9 millimols per liter and the p_H was 7.69. He became slightly disoriented and was given 1,000 cc. of physiologic solution of sodium chloride intravenously. On the eighth day the serum carbon dioxide was 49.2 millimols per liter and the p_H was 7.66; the blood urea nitrogen was 78 mg. per hundred cubic centimeters. Coma developed at this time, and the patient was given 400 cc. of physiologic solution of sodium chloride intravenously and 1,500 cc. subcutaneously. Muscular fibrillations appeared, and these were followed by a series of four convulsions. The blood pressure, which had been 160 systolic and 85 diastolic on entry, rose to 300 systolic and 125 diastolic and then gradually fell, first to 180 systolic and 110 diastolic and then to 95 systolic and 50 diastolic. Auricular fibrillation associated with severe cyanosis was noted at this time. The cardiac irregularity disappeared after the administration of digitalis. On the ninth day the serum carbon dioxide measured 43 millimols per liter, the p_H was 7.53, and the blood urea nitrogen was 90 mg. per hundred cubic centimeters. The blood pressure fluctuated from 125 to 135 systolic and from 80 to 85 diastolic. On the tenth day the serum carbon dioxide was 31.4 millimols per liter; the p_H was 7.50; the creatinine was 3.7 mg. and the blood urea nitrogen 98 mg. per hundred cubic centimeters. Death occurred on the tenth day as a result of cardiac failure. Several specimens of urine contained occasional red and white blood cells.

Necropsy revealed the following conditions: two chronic peptic ulcers and one healing superficial ulcer on the lesser curvature of the body of the stomach; cardiac dilatation, especially of the right chambers of the heart, with slight hypertrophy of the left ventricle; hypostatic hyperemia and edema of the lungs with acute bilateral bronchopneumonia; passive congestion of the liver with fatty changes; moderate generalized arteriosclerosis with sclerosis of the coronary arteries; focal myocarditis; encapsulated caseous and caseocalcareous tuberculosis of the right epididymus; healed calcified tubercles in the upper lobes of the left lung and in the left hilar lymph nodes and the peritracheal lymph nodes.

The remaining left kidney weighed 225 Gm. The capsule stripped easily, leaving a surface mottled with cyanotic and very pale areas; the cut surfaces

bulged. The cortex measured 8 mm. and was somewhat mottled with pale areas, in which the vascular markings were indistinct, and darker areas, where they were well preserved. The medulla was thinner than normal, and the tips of the medullary pyramids were fibrous. Microscopically, the glomeruli were within normal limits. The tubules were slightly dilated and contained much protein precipitate, occasional granular and hyaline casts, and leukocytes. A few collecting tubules contained a crystalline material staining faintly blue with hematoxylin and eosin, suggesting calcium. The epithelium of these tubules showed mild degenerative and regenerative changes. Surrounding these cortical tubules were scattered foci of round cell infiltration. The arterioles and larger-sized arteries showed some thickening.

CASE 3.—A 62 year old man had taken large amounts of sodium bicarbonate for many years for the relief of gastrointestinal distress, averaging 1 pound (373 Gm.) of alkali per week during the past two years. Sixteen months ago he had been hospitalized elsewhere for five months because of severe epigastric pain and vomiting. The symptoms persisted, and the patient entered the Albert Merritt Billings Hospital. His blood pressure was found to be 222 systolic and 116 diastolic. During the first six days he received 150 Gm. of sodium bicarbonate

TABLE 3.—*Acid-Base Balance in Case 3*

Hospital Day	Serum Cl, mM per Liter	Serum CO_2, mM per Liter	Serum p_H
4th	81.4	45.6	....
6th	70.0	57.1	7.50-7.58
7th	72.6	55.5	....
8th	78.0	58.8	....
9th	147.0 (?)	30.9	7.38

and calcium carbonate. On the seventh day he became drowsy and irrational and was given 1,000 cc. of physiologic solution of sodium chloride intravenously. The patient's condition grew progressively worse, and he lapsed into a coma on the ninth day. Solutions of 5 and 10 per cent dextrose in physiologic solution of sodium chloride were administered parenterally without avail, and the patient died in cardiac failure. Gastric aspirations during the first six days yielded from 700 to 1,000 cc. of gastric contents daily. Determinations of the acid-base balance (table 3) revealed severe alkalosis, which persisted until the ninth day. Renal function was not determined; one specimen of urine contained albumin.

Necropsy revealed a chronic benign ulcer in the prepyloric region of the stomach, acute pulmonary edema, acute distention of the right side of the heart, acute passive hyperemia of the liver and kidneys and moderate sclerosis of the abdominal aorta.

The kidneys were moderately enlarged and together weighed 350 Gm. They were of firm consistency. The capsules stripped easily, leaving smooth surfaces. The cortical markings were somewhat indistinct; the pyramids were cyanotic. The renal pelves and ureters were normal. Microscopic examination revealed many hyalinized glomeruli. Focal hemorrhage and thrombosis of the glomerular tufts were noted occasionally; these changes were not far advanced. There was considerable degeneration of the tubular epithelium associated with fatty degeneration. Rare tubules were lined by hyperplastic epithelium; a few eosinophilic and hyaline casts were seen. There was moderate scarring of the interstitial

tissue in the cortex, especially just below the capsule. No precipitation of calcium was observed. Some of the arterioles were markedly hyalinized, while others appeared relatively normal. The large arteries showed only moderate sclerosis.

CASE 4.—A 37 year old woman entered the hospital Jan. 18, 1935 with a history of distress from ulcers for five years. Alkalis had been taken occasionally during this time. The blood pressure was elevated, ranging from 154 to 170 systolic and from 88 to 110 diastolic. Seven hundred and forty-three grams of calcium carbonate and 826 Gm. of sodium bicarbonate were given in twenty-five days. Nine examinations of the urine gave negative results; the maximum specific gravity was 1.021. The blood urea nitrogen measured 11.3 mg. per hundred cubic centimeters, and the urea clearance was 65 per cent of average normal. Ulcer therapy was continued faithfully for the next year, and the patient remained fairly well except for frequent episodes of vomiting. She was readmitted April 10, 1936 because of massive hemorrhage. The treatment included parenteral adminis-

TABLE 4.—*Acid-Base Balance and Urea Clearance in Case 4.*

Hospital Day	Serum Cl, mM per Liter	Serum CO_2, mM per Liter	*pH*	Blood Urea N, Mg. per 100 Cc.	Urea Clearance, % Av. Normal	Ca, Mg. per 100 Cc.	P, Mg. per 100 Cc.
7th	....	62.1	7.65	....	..	...	...
8th	....	50.5	7.58	....	..	...	...
10th	....	36.0	7.53	....	..	...	...
14th	....	36.6	7.52	....	..	...	...
17th	83.7	37.4	7.52	....	..	...	...
21st	101.5	35.4	7.50	28.2	..	...	...
22d	....	....	....	25.4	22	...	...
28th	99.1	32.6	7.49	....	..	6.2	...
30th	....	....	....	....	..	7.5*	...
32d	....	....	....	95.0	52	...	...
35th	79.0	46.0	7.49	....	..	9.8	...
49th	....	....	....	....	..	9.9	3.9

* Plasma protein amounted to 6.4 Gm. per hundred cubic centimeters: albumin/globulin = 5.12/1.28 = 4.00.

tration of fluids and continuous aspiration of the stomach each night. Six hundred and seventy-four grams of calcium carbonate, 480 Gm. of sodium bicarbonate and 960 Gm. of tricalsate were given in forty-five days. Because of pyloric obstruction, gastroduodenostomy was performed on the thirty-ninth day, from which the patient made an uneventful recovery. Studies of the acid-base balance revealed severe alkalosis (table 4). On the twenty-seventh day a flexor spasm of both the forearms and the hands and numbness of the face developed; the Chvostek sign was positive. Three thousand cubic centimeters of 5 per cent dextrose in physiologic solution of sodium chloride was given parenterally, and the patient recovered promptly. It will be noted that the acid-base balance at this time was practically normal. The serum calcium was markedly lowered but returned to normal within seven days. Two of ten specimens of the urine contained a trace of albumin; the specific gravity ranged from 1.005 to 1.022. The patient took alkalis regularly for the next two years but entered the hospital a third time Dec. 21, 1937, thirty-five months after the initial visit, because of recurrent hemorrhage. She had been vomiting for nine days before admission. Three hundred and thirty grams of calcium carbonate and 900 Gm. of sodium bicarbonate were given in thirty-one days. The acid-base balance on the third day was normal; the blood urea nitrogen

measured 11.1 mg. per hundred cubic centimeters, and the urea clearance was 42 per cent of average normal. Eighteen days later there was a slight rise in the serum carbon dioxide (32 millimols per liter) and p_H (7.56); the blood urea nitrogen now measured 23.6 mg. per hundred cubic centimeters and the urea clearance 22 per cent. On the twenty-ninth day, however, the acid-base balance was normal; the blood urea nitrogen was 12.1 mg. per hundred cubic centimeters and the urea clearance 60 per cent. Seven examinations of the urine gave negative results; the specific gravity ranged from 1.008 to 1.018. Alkali therapy was continued subsequently for almost two years. The patient vomited frequently.

The fourth admission to the hospital occurred Nov. 5, 1940, five years and ten months after the original entry. The blood pressure at this time was 170 systolic and 94 diastolic. Wangensteen aspiration of the stomach was carried out for ten hours each day. Three thousand cubic centimeters of 5 per cent dextrose in distilled water was administered parenterally daily. This program was discontinued after five days, and on the seventh day salt was added gradually to the regimen. Eighty-eight grams of calcium carbonate was given in the last

TABLE 5.—*Acid-Base Balance in Case 4 (Subsequent Visit)*

Hospital Day.	Serum Cl, mM per Liter	Serum CO_2, mM per Liter	p_H	Blood Urea N, Mg. per 100 Cc.	Serum Ca, Mg. per 100 Cc.
2d	95.9	39.4	7.54	9.6	...
3d	....	....	....	21.5	...
5th	87.6	35.6	7.58	....	...
6th	86.8	35.1	7.61	....	...
7th	80.0	41.2	7.60	....	...
10th	56.7	41.3	7.47	18.6	...
11th	64.3	37.4	7.67	....	...
12th	80.1	....	....	9.4	...
14th	86.2	37.2	7.53	6.1	8.9
15th	....	....	....	8.8	...
16th	92.6	37.0	7.52	....	...

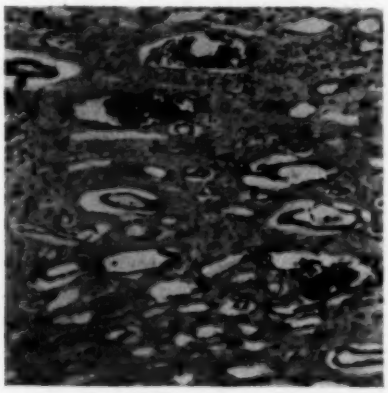
four days of this hospital period. A trace of albumin was noted in one of six specimens of the urine; the specific gravity ranged from 1.011 to 1.014. Numerous studies of the acid-base balance were made as shown in table 5.

The patient vomited frequently during the next month. She was readmitted for the last time Dec. 28, 1940 in a semicomatose condition. The reflexes were hyperactive, and there were coarse fibrillary muscular twitchings. The serum chloride measured 71 millimols per liter; the serum carbon dioxide, 48.5 millimols per liter. The p_H was 7.70. Fifteen hundred cubic centimeters of physiologic solution of sodium chloride was given parenterally to no avail. The patient had clonic convulsions and died several hours later.

Necropsy revealed an acute gastric ulcer with high grade stenosis of the pylorus, a chronic duodenal ulcer with high grade stenosis of an old gastroduodenostomy and perforation into the head of the pancreas, recent hemorrhage throughout the head of the pancreas and recent hemorrhage and edema of the lungs. Two parathyroid glands were normal grossly and histologically.

The left kidney weighed 100 Gm. The capsule stripped with moderate difficulty, leaving a diffusely granular pale surface. The cortex measured 4 to 6 mm. and the medulla 14 to 15 mm. The cortical rays were distinct; the pyramids were slightly hyperemic. The renal pelvis was normal; there was one recent subcapsular hemorrhage. The right kidney weighed 112 Gm. and was essentially similar in

appearance to the left kidney. Microscopic examination revealed an increase in fibrous tissue and a few fine cortical scars with foci of round cells near the capsule. A few glomeruli were completely hyalinized, and many others showed some degree of sclerosis, chiefly due to an increase in hyaline ground substance. In occasional glomeruli there was marked thickening of the basement membranes. The tubules varied considerably in appearance. Some were widened and lined by flat cuboidal cells; others had epithelium with hyperchromatic nuclei of varying size and form, and mitotic figures were seen occasionally, indicating active cell proliferation. There was extensive desquamation of the epithelium in some of the distal collecting tubules, and the lumens often were completely obstructed by loose epithelial cells and granular protein debris. Hyaline casts were few. Many collecting tubules were filled with coarse blue-staining or almost colorless granular masses which stained black with von Kossa's stain (fig.), indicating that they were composed of calcium. The epithelium of these tubules was necrotic. Foci of interstitial inflammation were noted surrounding the calcium-filled tubules. Some arterioles had thickened hyaline walls, but many of the vessels showed little change. The Gomori [8] stain revealed a markedly decreased alkaline phosphatase content of the kidney.



Photomicrograph demonstrating deposition of calcium in renal collecting tubules in case 4, group 3.

Although the kidneys in case 1 showed acute and chronic degenerative changes, most of the renal parenchyma was normal. Calcium precipitate was present in occasional collecting tubules. A few tubules were completely obstructed by these masses, and their epithelium presented mild degenerative and regenerative changes. Similar but less marked findings were noted in case 2, in which the alkalosis also had been severe. The kidneys in case 3 showed the typical findings of nephrosclerosis secondary to malignant hypertension. The changes in the tubules are attributable to the passive congestion. None of the findings could be related to the alkali therapy or to alkalosis. Case 4 was of great interest not only because the patient had taken large

8. Gomori, G.: J. Cell. & Comp. Physiol. **17**:71, 1941.

quantities of alkali for six years but also because there had been prolonged and excessive depletion of chloride through vomiting and gastric aspiration. She had experienced one episode of mild alkalosis and three bouts of severe alkalosis. Although considerable renal damage was visible, much of the parenchyma appeared fairly normal. Many of the changes, i. e., hyalinization of the glomeruli, tubular atrophy, increase of fibrous tissue and arteriolar thickening, are attributable to the hypertension which had been present. The precipitation of calcium in the lumens of the collecting tubules with obstruction of some of these tubules was more extensive in this case than in any other of the entire series. The histologic alterations resemble those of the interstitial pyelonephritis seen in patients not given alkalis; this possibility must be considered even in the absence of a suggestive history.

It is important to note that all patients of this group had vomited excessively and that all but 1 had had hypertension.

COMMENT

The most interesting histologic change in the kidney noted in this study was the calcium precipitate in the collecting tubules. Some of these tubules were obstructed by the calcium masses, and their epithelium showed both degenerative and regenerative changes. This finding was present in the kidneys of 3 patients who had previously experienced alkalosis but who did not die during the acid-base disturbance and in those of 3 patients in whom alkalosis was present at the time of death. All 6 patients had lost excessive amounts of chloride either by vomiting or by gastric aspiration.

The precipitation of calcium in the collecting tubules of the kidney is of considerable interest, although in the present study it did not appear to be of sufficient severity to destroy the function of more than relatively few nephrons. It is not a specific complication of "alkali alkalosis." It was first described by Nazari[9] in 1904 in 2 patients with vomiting secondary to pyloric stenosis. Many writers[10] subsequently emphasized its occurrence in persons with obstruction of the pylorus and of the upper part of the intestine, none of whom had received alkalis. It has been observed also in the kidneys of dogs after prolonged and severe

9. Nazari, A.: Policlinico (sez. med.) **11**:146, 1904.

10. (*a*) Brown, G.; Eusterman, G. B.; Hartman, H. R., and Rowntree, L. G.: Arch. Int. Med. **32**:425, 1923. (*b*) Zeman, F. D.; Friedman, W., and Mann, L. T.: Proc. New York Path. Soc. **24**:41, 1924. (*c*) Borst, J. G. G.: Ztschr. f. klin. Med. **117**:55, 1931. (*d*) Cooke, A. M.: Quart. J. Med. **2**:539, 1933. (*e*) Butler, A. M.; Wilson, J. L., and Farber, S.: J. Pediat. **8**:489, 1936. (*f*) Nicol, B. M.: Quart. J. Med. **9**:91, 1940. (*g*) Martz, H.: Arch. Int. Med. **65**:375, 1940.

depletion of chloride.[11] Similar changes have been noted by us after alkalosis secondary to continuous aspiration of gastric contents, as in the following case in which alkali had never been prescribed.

A 50 year old man entered the hospital with peritonitis following rupture of a gangrenous appendix. Because of abdominal distention, he was treated by continuous Wangensteen aspiration. Alkalosis developed despite the administration of saline solution, and the patient subsequently died of cardiac failure and bronchopneumonia. The blood chloride which had decreased to 214 mg. per hundred cubic centimeters rose to 436 mg. after the use of saline solutions; the carbon dioxide measured 28.3 millimols per liter, while the p_H varied from 7.54 to 7.59. The blood urea nitrogen was extremely high, measuring 231 and 256 mg. per hundred cubic centimeters; the blood creatinine was elevated to an unusual degree, measuring 6.75 and 7.0 mg. per hundred cubic centimeters. Microscopic examination of the kidneys revealed the presence of blue-staining material, probably calcium, in many of the collecting tubules. The epithelium of these tubules showed degenerative changes. There were numerous foci of interstitial inflammation; considerable protein debris as well as numerous casts were present in the lumens of the tubules. The histologic changes were identical with those observed in the 6 patients previously described.

The mechanism of this condition is not entirely clear. Calcium may be deposited in necrotic cells. The renal parenchyma often is normal, however, and the process may occur too rapidly for extensive necrosis to take place. Kerpel-Fronius and Martyn [12] produced salt deficiency in cats by ligation of the pylorus and noted calcium precipitation only in those animals in which both dehydration and alkalosis occurred; in the cats in which the development of alkalosis was prevented, only fatty degeneration of the tubules was observed. Hatano [13] was able to prevent this complication during experimental hypochloremia by the intravenous injection of sodium chloride, further emphasizing the importance of depletion of chloride in this process. The deposition of calcium in the kidney after alkalosis is due apparently to an alteration in the physical-chemical state of the urine resulting presumably in the precipitation of calcium phosphate and calcium carbonate. Enzymes, such as alkaline phosphatase, probably play no role in its development.[14] Zeman and associates [10b] studied the process in cats in which alkalosis was allowed to develop after ligation of the pylorus. The presence of calcium in the kidney was noted within forty-eight hours. Gömöri and Sarmai [15] later confirmed this observation and demonstrated that it was a reversible process, the calcium disappearing rapidly after removal of the pyloric obstruction.

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12. Kerpel-Fronius and Martyn, cited by Gömöri, P.; Podhradszky, L., and Kring, J.: Acta med. Scandinav. **102**:591, 1939.
13. Hatano, S.: Beitr. z. path. Anat. u. z. allg. Path. **102**:316, 1939.
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SUMMARY

No special histologic changes were noted in the glomeruli of the kidneys of 14 patients after prolonged alkali therapy with and without alkalosis. The epithelium of the collecting tubules in the kidneys of 6 patients showed degenerative and regenerative changes. There was precipitation of calcium in the lumens of these tubules. The tubules which did not contain calcium usually appeared normal. Three of these 6 patients had previously experienced alkalosis but did not die during the acid-base disturbance; alkalosis was present at the time of death in the other 3. All 6 patients had lost excessive amounts of chloride by vomiting or therapeutic aspiration. Similar changes were observed in a patient not receiving alkali but in whom severe hypochloremia and alkalosis developed as a result of gastric aspiration.

CONCLUSION

The long-continued administration of alkali in man with or without alkalosis does not lead to significant anatomic change in the kidneys attributable to alkali.

AMYLOID

III. THE PROPERTIES OF AMYLOID DEPOSITS OCCURRING IN SEVERAL SPECIES UNDER DIVERSE CONDITIONS

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In a recent study the end point of the alkaline solubility of the amyloid occurring in tissues of patients with chronic pulmonary tuberculosis was determined.[1] By taking advantage of the sharpness of this end point, solutions of amyloid that were not unduly contaminated with other soluble tissue components were obtained. From these solutions three fractions were isolated. The principal fraction was found to have the physical properties and elementary chemical constituents of a protein.[2] One minor fraction was identified as a sulfate-bearing polysaccharide.[2a] A second minor fraction has not been identified, although its physical behavior indicates that it is a protein.[1]

The designated studies were restricted to amyloid occurring in one species during the course of one disease. The present studies are more comprehensive and represent an inquiry into the question of variation in the composition of amyloid. This inquiry is concerned principally with relations between the solubilities of amyloid, the staining properties of the matrix and the apparent causes of amyloid disease in man, horses and rabbits.

MATERIAL AND METHODS

Species.—A study was made of tissues of man, horses, rabbits and mice.

Abnormal Conditions Serving as Apparent Causes of Amyloid Disease in This Study.—In this study amyloid disease was never encountered in an otherwise normal subject. For convenience the abnormal conditions which preceded and accompanied the development of the disease were regarded as noninfectious and infectious, singly or in combination.

The noninfectious abnormal conditions were of two types. One type was produced experimentally by successive injections of several antigens. The second type was produced by the disease known as plasma cell myeloma.

Horses, rabbits and mice were used in experiments in which antigens were injected. Diphtheria toxin, tetanus toxin, plain streptococcus toxin, scarlet fever

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From the Department of Pathology of Cornell University Medical College.

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TABLE 1.—*Relations Between the Method of Immunization of Horses, the Duration of Immunization, the Formation of Amyloid in Organs and the Iodine Reaction*

No.	Equine Organ Examined	Amount of Amyloid	Iodine Test		Immunization Data
9	Spleen	0	..	4/23/36 to 6/ 6/39	Agar mass
	Liver	+	±	7/23/36 to 1/30/40	Scarlet fever streptococcus toxin
	Kidney	0			
10	Spleen	+	±	5/26/36 to 2/ 4/38	Agar mass
	Liver	+	±	8/ 6/36 to 10/10/38	Plain streptococcus toxin
	Kidney	0	..	10/10/38 to 10/ 4/39	Scarlet fever streptococcus toxin
				10/ 4/39 to 1/30/40	Plain streptococcus toxin
11	Spleen	++	++++	8/18/38 to 3/ 6/40	Tetanus toxin
	Liver	++	++++	.....	Tetanus toxin + 0.5% alum
	Kidney	0			
12	Spleen	+	++	3/ 6/37 to 2/ 7/38	Plain streptococcus toxin
	Liver	+	++	2/14/38 to 11/ 2/38	Diphtheria toxin
	Kidney	0	..	11/ 2/38 to 3/11/40	Pneumococcus type XVIII (killed)
13	Spleen	++	++++	10/24/38 to 3/13/40	Tetanus toxin
	Liver	+++	++++	.....	Tetanus toxin + 0.5% alum
	Kidney	0			
14	Spleen	0	..	11/ 9/36 to 4/ 3/39	Diphtheria toxin
	Liver	+	±	.....	Diphtheria toxin + 0.3% $CaCl_2$
	Kidney	0	..	7/ 6/38 to 12/ 7/39	Pneumococcus type I (killed)
15	Spleen	++	+	11/ 9/36 to 11/ 2/38	Diphtheria toxin
	Liver	++	+	11/ 2/38 to 8/ 2/39	Pneumococcus type IV (killed)
	Kidney	0	..	10/ 9/39 to 4/13/40	Tetanus toxin
16	Spleen	+++	±	7/ 4/28 to 5/16/40	Tetanus toxin
	Liver	++++	±	.....	Tetanus toxin + 0.3% $CaCl_2$
	Kidney	..	..	.....	Tetanus toxin + 0.5% alum
17	Spleen	+	—	5/18/37 to 3/15/38	Diphtheria toxin
	Liver	+	—	.....	Diphtheria toxin + 0.3% $CaCl_2$
	Kidney	0	..	3/15/39 to 1/11/40	Plain streptococcus toxin; scarlet fever streptococcus toxin
18	Spleen	++	+++	7/13/37 to 1/ 5/39	Diphtheria toxin
	Liver	+++	++	4/23/38 to 4/25/40	Tetanus toxin
	Kidney	0	..	.....	Tetanus toxin + 0.3% $CaCl_2$; tetanus toxin + 0.5% alum
19	Spleen	0	..	4/ 7/37 to 5/31/38	Scarlet fever streptococcus toxin
	Liver	0	..	5/31/38 to 3/10/40	Tetanus toxin
	Kidney	0	..	.....	Tetanus toxin + 0.5% alum
20	Spleen	0	..	4/27/37 to 3/15/40	Pneumococcus type IV (killed)
	Liver	0			
	Kidney	0			
21	Spleen	0	..	3/10/37 to 3/14/40	Pneumococcus type IV (killed)
	Liver	0			
	Kidney	0			
22	Spleen	0	..	6/ 2/36 to 4/ 5/40	Pneumococcus type I (killed)
	Liver	0			
	Kidney	0			
23	Spleen	0	..	5/ 2/38 to 3/18/40	Pneumococcus type IV (killed)
	Liver	0			
	Kidney	0			
24	Spleen	0	..	8/ 1/36 to 8/20/36	Pneumococcus type I (killed)
	Liver	0	..	8/20/36 to 3/31/40	Pneumococcus type VII (killed)
	Kidney	0			
25	Spleen	0	..	2/ 1/38 to 4/16/40	Pneumococcus type XVIII (killed)
	Liver	0			
	Kidney	0			
26	Spleen	0	..	11/ 9/36 to 5/17/37	Diphtheria toxin
	Liver	0	..	.....	Diphtheria toxin + 0.3% $CaCl_2$
	Kidney	0	..	5/17/37 to 5/ 1/40	Pneumococcus type I (killed)
27	Spleen	0	..	7/17/39 to 1/10/40	Plain streptococcus toxin
	Liver	0	..	3/18/40 to 5/28/40	Diphtheria toxin + 0.5% $CaCl_2$
	Kidney	0			
28	Spleen	0	..	9/ 7/38 to 11/25/38	Diphtheria toxin
	Liver	0	..	11/25/38 to 6/25/40	Diphtheria toxin + 0.5% $CaCl_2$
	Kidney	0			
29	Spleen	0	..	20 years old	
	Liver	0	..	.....	No immunization
	Kidney	0			

streptococcus toxin and several types of heat-killed pneumococci were injected singly or in diverse combinations into 20 horses. The antigen or antigens administered to each horse and the periods of active immunization are listed in table 1.

Eight rabbits were given injections of several types of heat-killed pneumococci. Except for occasional five day intervals of rest, the injections were made intravenously three or four times each week over a period of four months.

Details of the methods used in the immunization of the horses and the rabbits need not be presented here. Suffice it to state that the animals were used for the production of immune serums under the direction of Dr. Jules Freund at the Research and Antitoxin Laboratory, Otisville, N. Y.[3]

Ninety-six young white mice were given injections of sodium caseinate. A fresh 5 per cent aqueous solution of this antigen was prepared every ten days by addition of the calculated quantity of sodium hydroxide to purified casein. The solution was sterilized by filtration and preserved at 5 C. in sterile tubes. Each mouse was given 0.25 cc. of the solution subcutaneously every five days. The experiment was terminated at the end of eight months. Forty-two mice lived throughout the experiment. Twenty-three mice which died during the experiment were likewise available for examination.

The second type of noninfectious condition which served as an apparent cause of amyloidosis was the disease plasma cell myeloma. A brief record of the only case in which material was obtained for study is as follows:

A man 39 years of age had been in good health until 1935, when he had an attack of acute arthritis. This subsided within a few weeks. In 1938 arthritis recurred and persisted. Many small and large joints were swollen and painful. As the arthritis progressed, limitation of motion of the joints increased, and firm tumor-like masses gradually developed in the subcutaneous tissues, especially around the larger joints. Laboratory tests disclosed severe progressive anemia, impairment of renal function and large quantities of protein in the urine. Several tests for Bence Jones protein in the urine showed none. The blood serum protein was always high. The maximum value was 9.5 Gm., with a serum globulin value of 7.3 Gm. and a serum albumin value of 2.2 Gm. per hundred cubic centimeters. The serum calcium value varied from 12.4 to 13.7 mg. and the serum phosphorus value from 3.6 to 4.9 mg. per hundred cubic centimeters.

Roentgenograms showed general osteoporosis with disseminated cystic areas of destruction of bone.

Biopsy of the tongue and of a subcutaneous nodule revealed masses of afibrillar homogeneous tissue, recognized as deposits of amyloid.

A differential count of cells in a specimen of the sternal marrow revealed a high percentage of plasma cells and led to a diagnosis of plasma cell myeloma.

The patient, aggravated by dysphagia and diminishing mobility of the tongue, gradually became weaker and died in September 1939, four years after the onset of symptoms of arthritis.

The postmortem examination established the diagnoses of plasma cell myeloma and amyloid disease. The kidneys, which contained large deposits of amyloid in the glomeruli, several subcutaneous periarticular masses of tissue which were composed almost exclusively of amyloid, and large blocks of skeletal muscle in which muscle fibers were replaced by amyloid, were preserved in the fresh state for study.

In the present investigation the only infectious disease which served as an apparent cause of amyloidosis was tuberculosis. Tissues were obtained from two

3. The tissues of horses and rabbits which had been immunized with pneumococci were received from the Research and Antitoxin Laboratory of the Department of Health of the City of New York, in Otisville, N. Y.

species, man and the rabbit. All the human subjects of amyloid disease, 21 in number, had chronic pulmonary tuberculosis due to the human tubercle bacillus. The fresh tissues were obtained post mortem through Dr. Reuben Schulz, of the

TABLE 2.—*Relations Between the Tuberculous Infection in Rabbits, the Duration of the Disease, the Superimposed Immunization, the Occurrence of Amyloid Disease and the Iodine Reaction of Amyloid Deposits*

No.	Rabbit Organ Examined	Amount of Amyloid	Iodine Test	Infection	Immunization
1	Spleen	++++	+	Bovine	None
	Liver	+	+	tubercle bacillus	
	Kidney	+++	+	(7 months)	
2	Spleen	++	++	Bovine	None
	Liver	0		tubercle bacillus	
	Kidney	++++	++	(10 months)	
3	Spleen	++	++	Bovine	None
	Liver	0		tubercle bacillus	
	Kidney	+++	++	(10 months)	
4	Spleen	+++	++++	Human	None
	Liver	+	+++	tubercle bacillus	
	Kidney	+++	+++	(12 months)	
5	Spleen	+++	—	Bovine	Heat-killed tubercle bacilli in liquid
	Liver	++	—	tubercle bacillus	petrolatum
	Kidney	++	—	(7 months)	
6	Spleen	+++	—	Bovine	Heat-killed tubercle bacilli in liquid
	Liver	++	—	tubercle bacillus	petrolatum
	Kidney	++	+++	(11 months)	
7	Spleen	++++	—	Bovine	Heat-killed tubercle bacilli in liquid
	Liver	++	—	tubercle bacillus	petrolatum
	Kidney	+++	+	(11 months)	
8	Spleen	++++	++	Bovine	Heat-killed tubercle bacilli in liquid
	Liver	+++	—	tubercle bacillus	petrolatum
	Kidney	++++	++	(12 months)	
9	Spleen	++++	..	Bovine	6 cc. tuberculin each week for
	Liver	++		tubercle bacillus	12 weeks during infection
	Kidney	+++		(3 months)	
10	Spleen	+++	..	Bovine	6 cc. tuberculin each week for
	Liver	++		tubercle bacillus	12 weeks during infection
	Kidney	++++		(3 months)	
11	Spleen	+++	..	Bovine	6 cc. tuberculin each week for
	Liver	++		tubercle bacillus	12 weeks during infection
	Kidney	+++		(3 months)	
12	Spleen	++++	..	Bovine	6 cc. tuberculin each week for
	Liver	++		tubercle bacillus	12 weeks during infection
	Kidney	++		(3 months)	
13	Spleen	0	..	Bovine	None
	Liver	0		tubercle bacillus	
	Kidney	0		(3 months)	
14	Spleen	0	..	Bovine	None
	Liver	0		tubercle bacillus	
	Kidney	0		(3 months)	
15	Spleen	0	..	Bovine	None
	Liver	0		tubercle bacillus	
	Kidney	0		(3 months)	
16	Spleen	0	..	Bovine	None
	Liver	0		tubercle bacillus	
	Kidney	0		(3 months)	

Middlesex Hospital, Cambridge, Mass., and Dr. Oscar Auerbach, of the Sea View Hospital, Staten Island, N. Y.

There were three groups of rabbits with tuberculosis and amyloid disease. These are listed in table 2. In the first group there were 4 animals. Three of these were infected by intravenous injections of bovine tubercle bacilli and the fourth by an intravenous injection of human tubercle bacilli. They lived for periods varying from seven to twelve months after onset of the infection. In the second

group of rabbits the bovine tuberculous infection was complicated by a course of immunization with heat-killed bovine tubercle bacilli suspended in liquid petrolatum. There were 4 rabbits in this series. They survived for periods varying from seven to twelve months. In the third group of rabbits the bovine tuberculous infection was complicated by weekly injections of 6 cc. of old tuberculin. In this experiment there were 8 animals. Four of these received injections of tuberculin during the three months' course of the tuberculous infection. The remainder, which served as controls, received the same dosage of tubercle bacilli but no injections of tuberculin. Tissues from these animals were obtained through Dr. Eugene Opie and his collaborators, Drs. Robert Huntington, Richard Linton and Newton Krumdieck.

EXAMINATION OF TISSUES

The study of amyloid deposits was routinely made on the spleen, the liver and the kidney. In the instance of the patient with plasma cell myeloma, the study was restricted to the kidney and to massive deposits of amyloid in the muscular and periarticular tissues. All tissues except those from the controlled series of tuberculous rabbits given tuberculin were available in the fresh state.

The fresh tissues were divided into two parts. One part was used for the study of the solubilities and morphologic properties of the amyloid. The second part was preserved in 4 per cent solution of formaldehyde for routine microscopic study in permanent sections.

The study of the solubilities and morphologic properties of amyloid was conducted as follows: Fresh blocks of tissue were cut into sections with a freezing microtome set at 20 to 25 microns. Sections of each organ or tissue were divided into four groups. One group was stained with compound solution of iodine U. S. P. (Lugol's solution) diluted so as to contain 0.1 per cent iodine, a second group with a 0.01 per cent aqueous solution of crystal violet and a third group with a 2 per cent aqueous solution of congo red. The fourth group was set aside for determinations of solubility. After making an immediate microscopic estimate of the amount of amyloid and of the quality of the staining reactions of the deposits, the residual unstained fresh sections were so divided that several sections were available for extraction with each of the following mediums: unbuffered aqueous fiftieth, seventy-fifth and hundredth normal solutions of sodium hydroxide, fifth normal solution of barium hydroxide, saturated solution of calcium hydroxide, saturated solution of strontium hydroxide and phosphate buffer solutions with p_H values of 10, 11, 11.2, 11.6 and 12. After sections were put in each solution, the flasks were sealed and kept at 5 C. At the end of twelve hours, three sections were removed from each flask, neutralized by washing successively in aqueous tenth-normal acetic acid and water and then stained with the standard solutions of iodine, crystal violet and congo red. The stained sections were studied microscopically at once. Estimates were made of the effects of the various solvents on the quantity and the staining properties of the amyloid. This procedure was repeated a second time after a total extraction period of twenty-four hours.

In addition to these routine studies there were several random experiments which were devised to test the action of buffer solutions of p_H values 1 to 10 on the deposits of amyloid in the different species.

RESULTS

All horses remained in good health during the courses of immunization by repeated injections of one or more noninfectious antigens. They

were killed while still in good health, and no evidence of infection was found at postmortem examination, though most of the horses had intestinal parasites. Amyloid deposits as recorded in table 1 were found. Inasmuch as most horses had been given injections of more than one type of antigen over different periods, as recorded in table 1, an exact analysis of the relations between the antigen or antigens and the characteristics of the amyloid deposits was impossible. Nevertheless, it was clear that all antigens were not equally effective in the production of amyloid disease. Tetanus toxin was most effective; the other antigens may be listed in order of decreasing effectiveness as follows: streptococcus toxins, diphtheria toxin and heat-killed pneumococci. None of the four types of pneumococci when used alone was capable of acting as an apparent cause of amyloidosis. The type of antigen was a more important factor in the production of amyloid disease in horses than the duration of the course of immunization beyond a minimum interval of time. Furthermore, there was no evidence that the increment in the amount of amyloid was principally dependent on prolongation of the minimum interval of time. For instance, in horse 13 severe amyloid disease developed during immunization with tetanus toxin over a period of eighteen months. Horse 19, treated in a similar way for twenty-one months, did not acquire amyloid disease, and horse 16, intensively immunized with tetanus toxin for twelve years, acquired but little more amyloid than the horse that had received the toxin for only eighteen months.

The only noninfectious antigens injected into normal rabbits were killed pneumococci. As in horses, these antigens were not effective in producing amyloid disease.

The only noninfectious antigen injected into mice was sodium caseinate. Despite prolongation of the period of immunization to eight months, the mice did not acquire amyloid disease.

So far as could be determined, the single case of plasma cell myeloma was not complicated by infection or stimulation of immune mechanisms by a noninfectious antigen. Nevertheless, as is occasionally observed in this disease, there was widespread amyloidosis. The distribution of the amyloid was unusual. The deposits were restricted principally to the walls of small arterioles, including those of renal glomeruli, to striated skeletal muscle and to periarticular or subcutaneous connective tissue. The intramuscular and periarticular deposits were especially abundant and often grossly resembled masses of hyaline cartilage. Microscopically, these masses were composed principally of homogeneous afibrillar tissue in which scattered fibrocytes were embedded.

In the present studies the tubercle bacillus was the only infectious agent which seemed to be responsible for the development of amyloid disease. This does not necessarily mean that the tubercle bacillus was the only infectious agent in the tissues. Other bacteria were often

present, especially in the chronic pulmonary lesions of several human beings with amyloid disease, but in the rabbits with experimental bovine tuberculosis superimposed nontuberculous infections were of little significance.

All the tuberculous infections were severe and prolonged, but in several human beings only one lobe of a lung was involved, and in 2 rabbits the tuberculous lesions were not conspicuous. The minimum duration of active tuberculosis was two years among the human subjects and seven months among the rabbits. The majority of the human subjects had had the disease over a period of many years. The incidence of amyloid disease in tuberculous rabbits increased rapidly in the second six month period of infection. Indeed, studies not reported here have revealed that in a large series of rabbits about 3 of every 4 animals had amyloid disease within fifteen months after onset of infection. Despite these findings, no consistent relation between the degree or the duration of tuberculosis and the quantity of amyloid in the tissue was found.

When rabbits with experimental bovine tuberculosis were given injections of an antigen, as recorded in table 2, amyloid deposits were often very prominent. We are not prepared to say whether the use of killed tubercle bacilli suspended in liquid petrolatum had any influence on the development of amyloid disease. However, it was clear that repeated injections of old tuberculin into animals during the course of a tuberculous infection led to the development of severe amyloid disease in three months. The control infected rabbits at the end of three months had about the same degree of tuberculosis but no evidence of amyloid disease. The injection of tuberculin into infected animals, therefore, not only hastened the onset of amyloid disease but also led to the rapid accumulation of a large quantity of amyloid in the tissues.

Though the results indicate that there is a species susceptibility to amyloidosis, adequately controlled studies will be required before the susceptibilities can be properly defined. Until such studies are undertaken, the following order of diminishing species susceptibility to amyloid disease may be proposed: rabbit, horse, man and mouse.

The quantitative distribution of amyloid in the spleen, the liver and the kidney differed among the several species. These differences are shown in tables 1, 2 and 8. In horses, amyloid deposits were more common in the spleen than in the liver, and no amyloid was found in the renal glomeruli. In rabbits the spleen always contained a larger amount of amyloid than the liver. Frequently, almost the entire structure of the spleen, especially the extrafollicular structure, was replaced by amyloid, while massive deposits were never found in the liver. As a rule, whenever amyloid was found in the spleen, it was likewise present in renal glomeruli and often in large amounts. In these instances the kidneys were enlarged, pale and usually the site of degenerative changes similar

to those described in amyloid nephrosis of human beings. When these renal changes were conspicuous, the impression was gained that they were responsible for the death of several animals, especially those with minimal tuberculosis. The distribution of amyloid in organs of the human subjects with tuberculosis, though not tabulated fully, was the same as that which is generally known. In severe disease, the deposits of amyloid were more voluminous in the liver than in the spleen, and the renal glomeruli were frequently normal even when 20 to 30 per cent of the spleen and the liver were replaced by amyloid. The susceptibility

TABLE 3.—*The Range of Solubility of Equine Amyloid in Phosphate Buffer Solutions at 5 C.*

No.	Equine Organ Examined	Amount of Amyloid	Iodine Test	Solubility of Amyloid at 5 C.						
				pH 10 (24 Hr.)	pH 11 (12 Hr.)	pH 11 (24 Hr.)	pH 11.6 (12 Hr.)	pH 11.6 (24 Hr.)	pH 12 (12 Hr.)	pH 12 (24 Hr.)
9	Spleen	0								
	Liver	+	±	0	++++	++++	++++	++++	++++	++++
	Kidney	0								
10	Spleen	+	±	0	++++	++++	++++	++++	++++	++++
	Liver	+	±	0	++++	++++	++++	++++	++++	++++
	Kidney	0								
11	Spleen	++	++++	0	+	++	+++	++++	++++	++++
	Liver	++	++++	0	+	++	+++	++++	++++	++++
	Kidney	0								
12	Spleen	+	++	0	++++	++++	++++	++++	++++	++++
	Liver	+	++	0	++++	++++	++++	++++	++++	++++
	Kidney	0								
13	Spleen	++	++++	0	+++	++++	++++	++++	++++	++++
	Liver	+++	++++	0	+++	++++	++++	++++	++++	++++
	Kidney	0								
14	Spleen	0								
	Liver	+	±	0	++++	++++	++++	++++	++++	++++
	Kidney	0								
15	Spleen	++	+	0	++++	++++	++++	++++	++++	++++
	Liver	++	+	0	++++	++++	++++	++++	++++	++++
	Kidney	0								
16	Spleen	+++	±	0	+	+	++	++	++++	++++
	Liver	++++	±	0	+	+	++	++	++++	++++
	Kidney	0								

of organs of mice was not disclosed since amyloidosis did not develop during the eight month period of injection of sterile sodium caseinate.

The aniline dye reactions of amyloid were constant, while the iodine reaction was variable. There was no significant variation in the intensity or the quality of the congo red or the crystal violet reactions among the several amyloid deposits. On the contrary, variations of the intensity of the iodine reaction were encountered. The variations as judged microscopically are recorded in tables 1, 2 and 8. In general, the reaction was strongly and uniformly positive only in instances of human amyloidosis. Similar strong reactions occurred in 2 instances of equine amyloidosis and 1 instance of amyloid disease in rabbits. The 2 horses in which strongly reactive amyloid was found had received the same antigen, tetanus toxin. The rabbit in which the strongly reactive amyloid was found was the only rabbit that was infected with the human tubercle

bacillus. As a rule, however, the iodine reaction was weak or negative in horses or rabbits and was not influenced by the duration of the disease, the quantity of amyloid or the solubility of the amyloid (tables 1 to 8).

TABLE 4.—*Relations Between the Quantity of Amyloid in Tissues of a Patient with Multiple Myeloma, the Intensity of the Iodine Reaction, and the Range of Solubility in Buffer Solutions*

Number	Human Myeloma Amyloid	Amount of Amyloid	Iodine Test	Observation at 24 Hours at 5 C.									
				p_H 1-10		p_H 11		p_H 11.2		p_H 11.6		p_H 12	
				Solubility	Iodine Test	Solubility	Iodine Test	Solubility	Iodine Test	Solubility	Iodine Test	Solubility	Iodine Test
100	Intramuscular	++++	++++	0	++++	0	++++	0	++	+++	0	++++	0
100	Periarticular	++++	++++	0	++++	0	++++	0	++	+++	0	++++	0
100	Renal	+++	++++	0	++++	0	++++	0	++	++	0	++++	0

TABLE 5.—*Relations Between the Quantity of Amyloid in Organs of Tuberculous Rabbits, the Initial Intensity of the Iodine Reaction and the Solubility of the Amyloid in Phosphate Buffer Solutions*

No.	Rabbit Organ Examined	Amount of Amyloid	Iodine Test	Solubility of Amyloid at 5 C.						
				p_H 10 (24 Hr.)	p_H 11 (12 Hr.)	p_H 11 (24 Hr.)	p_H 11.6 (12 Hr.)	p_H 11.6 (24 Hr.)	p_H 12 (12 Hr.)	p_H 12 (24 Hr.)
1	Spleen	++++	+	0	0	0	+	++	+++	+++
	Liver	+	+	0	0	0	++	+++	++++	++++
	Kidney	+++	+	0	0	0	+	++	+++	+++
2	Spleen	++	++	0	0	0	+	++	+++	+++
	Liver	0								
	Kidney	++++	++	0	0	0	+	++	+++	+++
3	Spleen	++	++	0	0	+	++	+++	+++	++++
	Liver	0								
	Kidney	+++	++	0	0	+	++	+++	+++	++++
4	Spleen	+++	++++	0	+	+	++	++	++++	++++
	Liver	+	+++	0	0	+	++	+++	++++	++++
	Kidney	+++	+++	0	+	+	++	++	++++	++++
5	Spleen	+++	0	0	0	0	++	++	++++	++++
	Liver	++	0	0	0	0	++	++	++++	++++
	Kidney	++	0	0	0	0	++	++	++++	++++
6	Spleen	+++	0	0	++	++	+++	+++	++++	++++
	Liver	++	0	0	++	++	+++	+++	++++	++++
	Kidney	++	+++	0	++	++	+++	+++	++++	++++
7	Spleen	++++	0	0	0	0	++	++	++	++++
	Liver	++	0	0	0	0	++	++	+++	++++
	Kidney	+++	+	0	0	0	++	++	++	++++
8	Spleen	++++	++	0	0	0	++	++	+++	++++
	Liver	+++	0	0	0	0	++	++	+++	++++
	Kidney	++++	++	0	0	0	++	++	+++	+++

The several amyloids did not have the same solubility. The various solubilities are listed in tables 3 to 8. No amyloid which was soluble anywhere in the range p_H 1 to 10 has yet been encountered. All amyloid matrices were soluble somewhere in the range p_H 11 to 12. The amyloid in the human beings with chronic pulmonary tuberculosis was always soluble at the lower limit of this range. Usually, as shown in table 3, the

amyloid in horses was soluble at this lower limit, but in 1 instance a solution with p_H 11.6, and in another instance a solution with p_H 12, was necessary for dissolving the amyloid matrix. The amyloid in rabbits

TABLE 6.—*Relations Between the Quantity of Amyloid in Organs of Tuberculous Rabbits, the Initial Intensity of the Iodine Reaction and the Solubility of the Amyloid in Alkaline Unbuffered Solutions*

				Solubility of Amyloid at 5 C. in 24 Hours					
No.	Rabbit Organ Examined	Amount of Amyloid	Iodine Test	N/100 NaOH	N/75 NaOH	N/50 NaOH	N/5 $Ba(OH)_2$	$Ca(OH)_2$ (Saturated)	$Str(OH)_2$ (Saturated)
8	Spleen	++++	++	0	++	++++	0	0	+
	Liver	+++	0	0	++	++++	0	0	0
	Kidney	++++	++	0	++	++++	0	0	+
4	Spleen	+++	++++	0	+++	++++	0	0	+
	Liver	+	+++	0	+++	++++	0	0	—
	Kidney	+++	+++	0	+++	++++	0	0	+
6	Spleen	+++	±	0	+++	++++	0	0	0
	Liver	++	—	0	++	++++	0	0	0
	Kidney	++	+++	0	++	++++	0	0	0
7	Spleen	++++	±	0	++	++++	0	0	+
	Liver	++	0	0	+	++++	0	0	+
	Kidney	+++	+	0	++	++++	0	0	+

TABLE 7.—*Relations Between the Amount of Amyloid in Organs of Immunized Horses, the Initial Intensity of the Iodine Reaction and the Solubility of the Amyloid in Alkaline Unbuffered Solutions*

				Solubility of Amyloid at 5 C. in 24 Hours					
No.	Equine Organ Examined	Amount of Amyloid	Iodine Test	N/100 NaOH	N/75 NaOH	N/50 NaOH	N/5 $Ba(OH)_2$	$Ca(OH)_2$ (Saturated)	$Str(OH)_2$ (Saturated)
9	Spleen	0							
	Liver	+	±	++	++++	++++	++++	++++	++++
	Kidney	0							
10	Spleen	+	±	0	++++	++++	++++	++++	++++
	Liver	+	±	0	++++	++++	++++	++++	++++
	Kidney	0							
11	Spleen	++	++++	0	+++	++++	+	++	++
	Liver	++	++++	0	+++	++++	+	++	++
	Kidney	0							
12	Spleen	+	++	0	++++	++++	++++	++++	++++
	Liver	+	++	0	++++	++++	++++	++++	++++
	Kidney	0							
13	Spleen	++	++++	0	+++	++++	++	+	+++
	Liver	+++	++++	0	+++	++++	++	+	+++
	Kidney	0							
14	Spleen								
	Liver	+	±	0	++++	++++	++++	++++	++++
	Kidney	0							
15	Spleen	++	+	0	++++	++++	++	0	++++
	Liver	++	+	0	++++	++++	++	0	++++
	Kidney	0							
16	Spleen	+++	±	0	++++	++++	++	+	+++
	Liver	++++	±	0	++++	++++	++	+	+++
	Kidney	0							

and that in the patient with plasma cell myeloma were still more resistant to the solvent action of the buffer solutions, the matrices being never completely dissolved at 5 C. in solutions less alkaline than p_H 12 (tables 4 and 5). In the correlative analysis of these variable data the conclusion

TABLE 8.—*Relations Among the Solubilities and Iodine Reactions of Amyloid Produced in Several Species by Different Apparently Causative States*

Species	Condition Apparently Related Etiologically to Amyloidosis	Organ Examined	Amount of Amyloid	Iodine Test	p_H 10		p_H 11		N/5 $Ba(OH)_2$		Saturated $Ca(OH)_2$	
					Solubility	Iodine Test	Solubility	Iodine Test	Solubility	Iodine Test	Solubility	Iodine Test
Man	Tuberculosis	Liver	+++	++++	0	++++	++++	—	0	—	0	—
Man	Tuberculosis	Liver	+++	++++	0	++++	++++	—	+	—	0	—
Man	Tuberculosis	Liver	++	++++	0	++++	++++	—	0	+	0	+
Man	Myeloma	Muscle	+++	++++	0	++++	0	++++	0	+	0	+
Horse	Tetanus toxin	Spleen	++	++++	0	++++	++	+	+	+	++	—
Horse	Tetanus toxin	Liver	++	++++	0	++++	++	+	+	+	++	—
Horse	Tetanus toxin	Spleen	++	++++	0	++++	++++	—	++	—	+	—
Horse	Tetanus toxin	Liver	+++	++++	0	++++	++++	—	++	—	+	—
Horse	Several toxins	Spleen	+	++	0	++	++++	—	++++	—	++++	—
Horse	Several toxins	Liver	+	++	0	++	++++	—	++++	—	++++	—
Horse	Diphtheria and tetanus toxins	Spleen	++	+++	0	+++	+++	+	++	—	+	—
Horse	Diphtheria and tetanus toxins	Liver	+++	++	0	++	+++	+	++	—	+	—
Rabbit	Tuberculosis	Spleen	+++	++++	0	++++	+	+	0	++	0	+
Rabbit	Tuberculosis	Liver	+	+++	0	+++	+	—	0	+	0	+
Rabbit	Tuberculosis	Kidney	+++	+++	0	+++	+	—	0	+	0	+
Rabbit	Tuberculosis	Spleen	++	++	0	++	0	—	0	—	0	—
Rabbit	Tuberculosis	Kidney	++++	++	0	++	0	—	0	—	0	—
Rabbit	Tuberculosis	Kidney	++	+++	0	+++	++	+	0	+	0	—
Rabbit	Tuberculosis	Spleen	++++	+	0	+	0	—	0	—	0	—
Rabbit	Tuberculosis	Liver	+	+	0	+	0	—	0	—	0	—
Rabbit	Tuberculosis	Kidney	+++	+	0	+	0	—	0	—	0	—
Rabbit	Tuberculosis	Kidney	++++	++	0	++	0	—	0	+	0	—

was drawn that constant solubility was the rule when the variables of species and apparent cause were fixed. There was no correlation between the solubility of amyloid and the duration of the amyloidosis, the quantity of amyloid or the intensity of the iodine reaction.

The differences in solubility of amyloid, as disclosed by use of buffered solutions, were again illustrated by use of alkaline unbuffered solutions as solvents (tables 6, 7 and 8). In aqueous sodium hydroxide solution every amyloid was soluble at some concentration in the range hundredth to fiftieth normal. In general, the types of amyloid which dissolved at p_H 11 were soluble at the lower alkaline limit of this range, and those which dissolved at p_H 12 were soluble only at the upper alkaline limit of the range. Other alkaline solutions had variable actions. As a rule, strontium hydroxide was more effective as a solvent than barium hydroxide, and calcium hydroxide was the least effective. These reagents did not dissolve the amyloid of tuberculous rabbits or, as shown in other studies, the amyloid occurring in human beings with tuberculosis (tables 6 and 8). They proved to be moderately efficient as solvents for the amyloid in immunized horses, especially when the quantity of amyloid in the tissues was small (table 7).

Irrespective of the nature of the solvent, the affinities of the amyloid matrix for crystal violet and congo red persisted until the last trace of amyloid was dissolved. On the contrary, the iodine reaction which initially was positive often became negative in the presence of various reagents, even though the amyloid matrix otherwise exhibited no significant change (tables 4 and 8). In all instances the intensity of the iodine reaction at neutrality was fully maintained throughout the range p_H 7 to 10. Positive iodine reactions usually became negative during extraction of the tissue with aqueous fifth-normal barium hydroxide solution, a saturated solution of calcium hydroxide or a phosphate buffer solution of p_H 11. The loss of the capacity of the amyloid matrix to combine with iodine to give a positive reaction after treatment with these reagents was not specifically related to the solubility of the entire matrix or to any other factor which could be discovered.

COMMENT

These studies show some relationships between abnormal conditions which served as apparent causes of amyloid disease, the species in which the disease occurred, the solubilities of the matrix and the staining qualities of the matrix.

Chronic destruction of tissue with repair was common to all apparent etiologic states. This characteristic was especially prominent in most rabbits with experimental tuberculous infections, in human beings with pulmonary tuberculosis and in the patient with plasma cell myeloma. In other instances, especially those in which amyloidosis developed in

horses during courses of active immunization, the degree of tissue injury was minimal and was localized to parenteral sites of injections of antigens. If tissue injury with repair had anything to do with the development of amyloid disease, it is certain that the degree of injury was not of first importance. Likewise, the duration of the tissue injury beyond a variable minimum limit of time had no consistent quantitative bearing on the development and progress of amyloidosis. This variable interval of time between the onset of the abnormal state and the first appearance of amyloid in the tissues depended on at least two factors. One factor was inherent in the species, for the interval of time was shortest with rabbits, of medium length with horses and of greatest length with human beings. A second factor was the nature of the abnormal condition which served as an apparent cause of amyloidosis. For example, rabbits with tuberculosis usually acquired amyloid disease within a period of seven to twelve months. In the experiment in which the tuberculous rabbits were given subcutaneous injections of tuberculin, amyloid disease was severe at the end of three months. In the control rabbits, which had a similar amount of tuberculosis and which received no tuberculin, amyloidosis did not develop during the three month period of infection. Thus, the usual course of development of amyloid disease in tuberculous rabbits was shortened and the quantity of amyloid deposited was increased by subcutaneous injections of tuberculin. This effect of tuberculin in rabbits is in accord with the well known observation that in horses repeated injections of certain antigens lead to the development of amyloid disease in the absence of infections or significant injury of tissue.[4]

It seems, therefore, that persistent or repeated stimulation of immune mechanisms is a fundamental factor in the genesis of amyloid disease. This factor was common to all except 1 of the instances of amyloid disease which we have studied. The exception was the case of plasma cell myeloma, and other exceptions have been described in the literature. In these instances there has been no proof that immune mechanisms had not been actively stimulated, and until such proof is offered they cannot logically be regarded as true exceptions to the rule.

A more positive argument against the fundamental importance of antigenic stimulation in the genesis of amyloid disease is the frequent absence of the disease in the presence of intensive prolonged stimulation of immune mechanisms. This raises the question of the qualitative and quantitative aspects of immunity. So far as qualitative aspects are concerned, the present studies illustrate conspicuous differences in effectiveness among antigens. In horses, tetanus toxin was most effective in the production of amyloid disease, while killed pneumococci were not effective. The ineffectiveness of killed pneumococci was again illustrated by experiences with rabbits, all of which received many injections over a

4. Arndt, H. J.: Verhandl. d. deutsch. path. Gesellsch. **26**:243, 1931.

period of four months without the development of amyloidosis.[5] Likewise, mice which were given injections of a solution of sterile sodium caseinate did not acquire the disease. Despite the fault of comparing the effects of different antigens in species of different susceptibilities, the conclusion may be justified that in a suitably controlled experiment antigens with different qualities would have different capacities for producing conditions leading to amyloid disease. From the quantitative aspect of immunity the more important theoretic considerations would be the level of the serum antibody titer, the quantity of circulating antigen, the insolubility of the antigen-antibody precipitate and the length of the period of immunization.[6] It remains for experiments to show whether or not there are precise correlations between these factors and the occurrence of the disease.

The implied order of diminishing species susceptibility to the disease, namely, rabbit, horse, man and mouse, may be more apparent than real since the conditions were not the same among the several species. If this is a true order of susceptibility, the experimental data offered no explanation.

The order of susceptibility of organs among the several species may be regarded as a more reliable result of this study. Except for the instance of atypical amyloidosis complicating plasma cell myeloma, the order was fairly constant in each species and was independent of variables such as apparent causative condition and duration of the amyloidosis. The interesting studies of Dick and Leiter [7] indicate that there are exceptions to this general conclusion and that resorption of amyloid is an important factor in the regulation of the observed distribution in organs. The most conspicuous examples of susceptibility of organs were the massive deposits of amyloid in the spleens of many tuberculous rabbits and the absence of amyloid deposits in the renal glomeruli of horses. The latter observation was surprising because in man and especially in rabbits glomerular arterioles are elective sites for the deposition of amyloid. It is possible that the species variation in arteriolar dimensions has something to do with the observed differences.

The variations in the solubility of amyloids were independent of any apparent factor. As a rule, with a given abnormal state in a given species the solubility was constant and was independent of the duration of the abnormal state, the quantity of amyloid or the intensity of the iodine reaction. But with different abnormal states in a given species, as illustrated by the equine and the human material, the amyloid deposits

5. Repeated injections of living pneumococci of type III, however, will lead to the onset of amyloid disease in rabbits within a period of eight months (Angevine, D. M., and Freund, J.: Personal communication to the authors).

6. Letterer, E.: Virchows Arch. f. path. Anat. **293**:34, 1934. Eklund, C. M., and Reimann, H. A.: Arch. Path. **21**:1, 1936.

7. Dick, G. F., and Leiter, L.: Am. J. Path. **17**:741, 1941.

had different solubilities. Though these differences were not conspicuous, they were detectable and offer some support to the thesis that amyloid is a product of variable composition.

The concept that amyloid may have a variable composition is in agreement with the differences in the intensity of the iodine reaction. The differences which were encountered in the present study could not be correlated satisfactorily with any special factor. Only human amyloid consistently gave a strong positive reaction. In the amyloid of rabbits and horses the reaction was usually weakly positive or negative. If these variations in the reaction are eventually to be explained by the presence of some compound in the amyloid matrix, it may be inferred from the data that the reactivity of the compound can be modified without producing any detectable quantitative change in the matrix or qualitative change in the affinities of the matrix for crystal violet and congo red. In view of the experimental modifications of the quality of the reaction in vitro, doubt remains as to whether the induced changes in the reaction were due to a change in some compound which remained in the matrix or to removal of some minor component by solvents which did not dissolve the principal part of the matrix. If some compound in the matrix was changed, the change was irreversible. If some minor component was removed from the matrix, it is possible that the component was the sulfate-bearing polysaccharide recently isolated from amyloid. Though possible, it is unlikely that extraction of the polysaccharide was responsible for the loss of the iodine reaction, since the polysaccharide is not extractable under some conditions which lead to a change of the iodine reaction. Though this problem has not been solved, a first step in the solution has been made by specification of methods by which the iodine reaction and the principal part of the matrix may be modified in a differential way with several alkaline reagents. If possible, at least for chemical purposes, complementary methods for the differential modification of the matrix and the iodine reaction with acid reagents should be made available.

Though all types of amyloid disease were not studied, the data define several simple properties of amyloid in special cases. The definition of these properties may facilitate a more complete analysis of the composition of amyloid in man and two species of experimental animals. Furthermore, the data offer standards by which the different amyloids may be crudely distinguished from one another and from morphologically similar hyaline intercellular substances. Among these similar substance the most common are those which occur in the vascular disease known as arteriolosclerosis. As these have not been defined by other than morphologic methods, it is possible that some of the confusion which centers about differences between hyaline and amyloid "degeneration" can be dispelled by appropriate studies of the solubilities of the products in

question.[8] This would seem to be the simplest and most direct approach to the objective of segregation of the several products in purified form and of final analysis of their genesis.

SUMMARY

A comparative study of amyloid disease in man, horses and rabbits was made. In man the apparent cause of the disease was chronic pulmonary tuberculosis or plasma cell myeloma; in horses, it was prolonged immunization with one or more noninfectious antigens, and in rabbits, chronic tuberculosis, which in several animals was complicated by injections of heat-killed tubercle bacilli or old tuberculin. Amyloid disease did not develop in horses and rabbits during prolonged immunization with heat-killed pneumococci, nor could the disease be produced in mice by repeated injections of sterile sodium caseinate.

Amyloid disease developed more readily in rabbits than in horses or in man. The most effective method of producing the disease in rabbits was that of injecting tuberculin into animals that had active bovine tuberculosis. The most effective method of producing it in horses was that of injecting tetanus toxin. In a given species the quantity of amyloid depended primarily on the nature of the apparent causative condition and secondarily on the duration of this condition.

There was a species variation in the quantitative partition of amyloid in the spleen, the liver and the kidney. Splenic amyloid was most voluminous in rabbits. Hepatic amyloid was most prominent in man. Renal amyloid was more abundant in rabbits than in man and was never found in horses.

The solubility of different amyloid matrices varied. All were soluble somewhere in the range p_H 11 to 12. Various ones had different solubilities in solutions of barium, calcium and strontium hydroxide. The solubilities were constant in a given species in the presence of a given apparently causative condition, and they did not vary with the age of the amyloid deposits, the quantity of amyloid or the intensity of the iodine reaction.

All amyloid deposits gave similar aniline dye reactions, but the intensity of the iodine reaction varied. Human amyloid always gave a near maximum positive iodine reaction, while the reaction of amyloid in horses and rabbits was usually weak or negative. The intensity of a positive reaction was not diminished during extraction of amyloid at p_H 10, but positive reactions became negative at p_H 11 or during extraction with barium, calcium or strontium hydroxide. The change from positive to negative reactions often occurred, especially during extraction with calcium hydroxide, without any appreciable change in the quantity of amyloid or the affinities of the matrix for crystal violet and congo red.

8. Leupold, E.: Ergebn. d. allg. Path. u. path. Anat. (pt. 1) **21**:120, 1925.

THE VENOUS VALVES IN THROMBOANGIITIS OBLITERANS

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It is well established that thromboangiitis obliterans attacks the veins of the extremities as extensively as it does the arteries. It would be indeed surprising if the delicate valves of the veins should escape involvement of some kind. Yet in the extensive literature on thromboangiitis obliterans we have found only a parenthetic mention of valvular changes.[1]

In previous publications we have demonstrated that in ordinary thrombophlebitis [2] and in varicose veins [3] there is extensive involvement of the valves, which may account for much of the disturbance of the venous return. We may similarly infer that if damage of the valves were demonstrated in thromboangiitis obliterans it would help to explain some of the circulatory difficulties in this disease.

Accordingly, we have studied the blood vessels of lower extremities from a series of patients with thromboangiitis obliterans and have observed that the venous valves are seriously damaged.

In order that the cases included here may be accepted as authentic instances of thromboangiitis obliterans, we shall indicate what criteria we have used in making the diagnosis. The absolute microscopic criterion of thromboangiitis obliterans is the presence of an intraluminal granuloma consisting of fibroblasts, lymphocytes, macrophages, and multinucleated giant cells resembling the foreign body type (fig. 1). This lesion may most often be found localized in segments of the superficial veins. It is met with uncommonly in the deep veins and rarely in the arteries. But the vessels lacking this pathognomonic tissue often show fibromuscular intimal thickening or thrombosis, while some segments show no evident abnormality. The diagnosis is strongly indicated when there is thrombosis of both arteries and veins with fibrosis of the

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1. von Winiwarter, F.: Arch. f. klin. Chir. **23**:202, 1879. Popoff, N. W.: Arch. Path. **18**:295, 1934.
2. Edwards, E. A., and Edwards, J. E.: Surg., Gynec. & Obst. **65**:310, 1937.
3. Edwards, J. F., and Edwards, E. A.: Am. Heart J. **19**:338, 1940.

perivascular tissue, often including the accompanying nerves. Infiltration of macrophages in all layers of the vein, together with reduplication of the elastic laminas, and scarring, with interruption of the mural structure, are also very suggestive.

In the absence of the specific granulomatous lesions, the clinical status is a great aid in establishing the diagnosis. Classically, one finds evidence of arterial insufficiency in a man under 40 who is a heavy smoker of tobacco, and who shows evidence of migrating phlebitis or gives a history of this condition. In the absence of the specific changes,

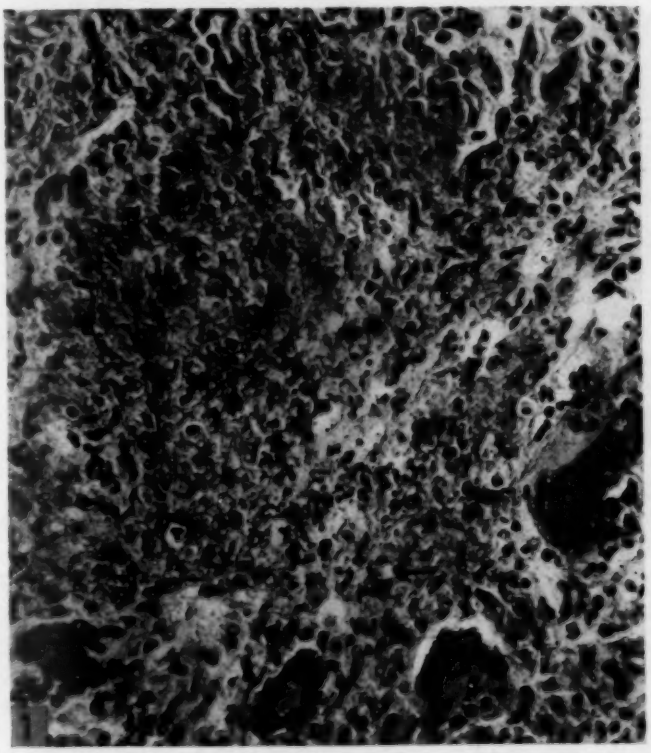
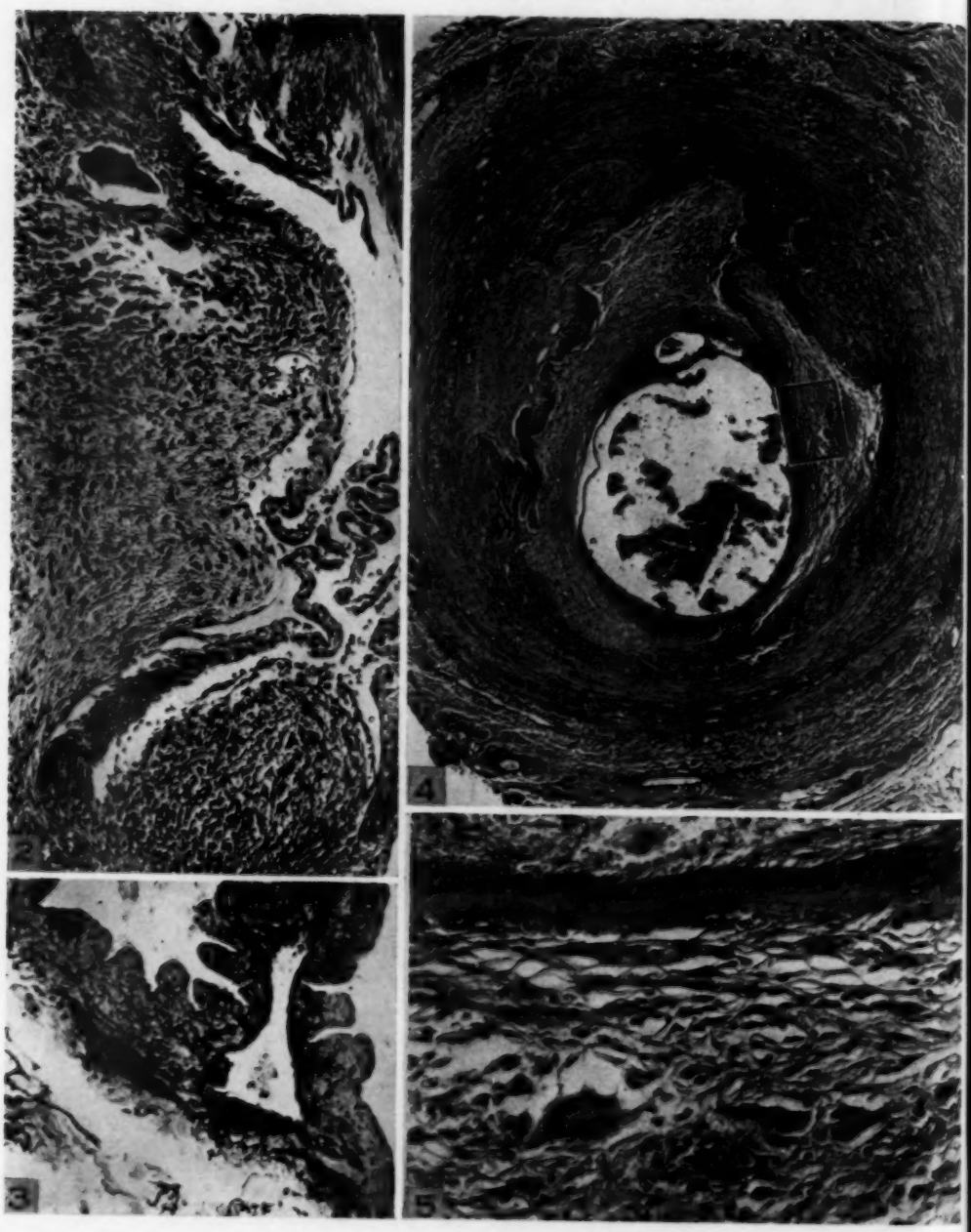


Fig. 1.—The intraluminal granuloma of thromboangiitis obliterans. Besides the multinucleated giant cells, there are numerous macrophages, fibroblasts and lymphocytes. A central area of necrosis is present. × 310.

the association of such circumstances with the aforementioned suggestive pathologic changes makes almost certain the diagnosis of thromboangiitis obliterans.

The blood vessels used in this study were obtained from 23 amputated extremities and 3 bodies on which autopsies had been done.[4] The

4. Dr. Tracy B. Mallory gave us the opportunity to include in our study the material from the department of pathology of the Massachusetts General Hospital, and Dr. Richard Wadsworth the material from a patient at the Metropolitan State Hospital, Waltham, Mass.



Figures 2 to 5

(See legends on opposite page)

photomicrographs shown here were made from the vessels obtained in 9 cases. In 5 instances the specific lesion was present; in the following paragraphs will be found the significant clinical features in the remaining 4 cases.

Case 1.—A man admitted to the Boston City Hospital stated that the onset of pain in the extremities occurred when he was 30 years old. He smoked cigarets, but the number consumed is not recorded. In the twelve years between the first symptoms and his appearance in the hospital he had submitted to amputation successively of the left great toe, the remaining toes, part of the left foot, the lower third of the leg, the middle third of the leg, the lower part of the left thigh and finally the middle of the left thigh.

During this time he had begun to have pain in the right foot. Examination showed no pulsation below the level of the femoral artery. The urine showed no albumin or sugar; the Hinton test of the blood was negative. A series of sensory nerve crushings had been done with some relief, and this was followed by lumbar sympathectomy on the right side. He improved and was promptly discharged. He returned nine months later because of an ulcer of the foot. This progressed, and the right leg was amputated at the junction of its middle and upper thirds. The tissue examined in this study was obtained from this limb.

Case 2.—A man entered the Boston City Hospital at 34 years of age. He smoked cigarets, but the number consumed is not recorded. He had had pain and claudication for six months. There were no pedal pulses, but the popliteal artery pulsated. Cyanosis was marked. In the course of five days his condition progressed to frank gangrene and midthigh amputation was done on the left extremity. Examination of the vessels showed old thrombosis of the posterior tibial artery with recent thrombosis of the popliteal artery.

He returned four months later because of thrombophlebitis of the right saphenous vein. The foot was pale and perspiring. While under observation the femoral vein was likewise involved in thrombophlebitis. A right lumbar sympathectomy was done, and the patient was discharged improved. He came back ten months after his second admission because of pain in the right great toe. The toe was swollen, purple and cold. There were no pulses below the femoral level. The Hinton test of the blood was "doubtful." The urine showed no sugar. He failed to improve after conservative treatment, and a midthigh amputation was done. Old and recent thrombosis of the posterior tibial vessels was found. The vessels of this limb were included in the present study.

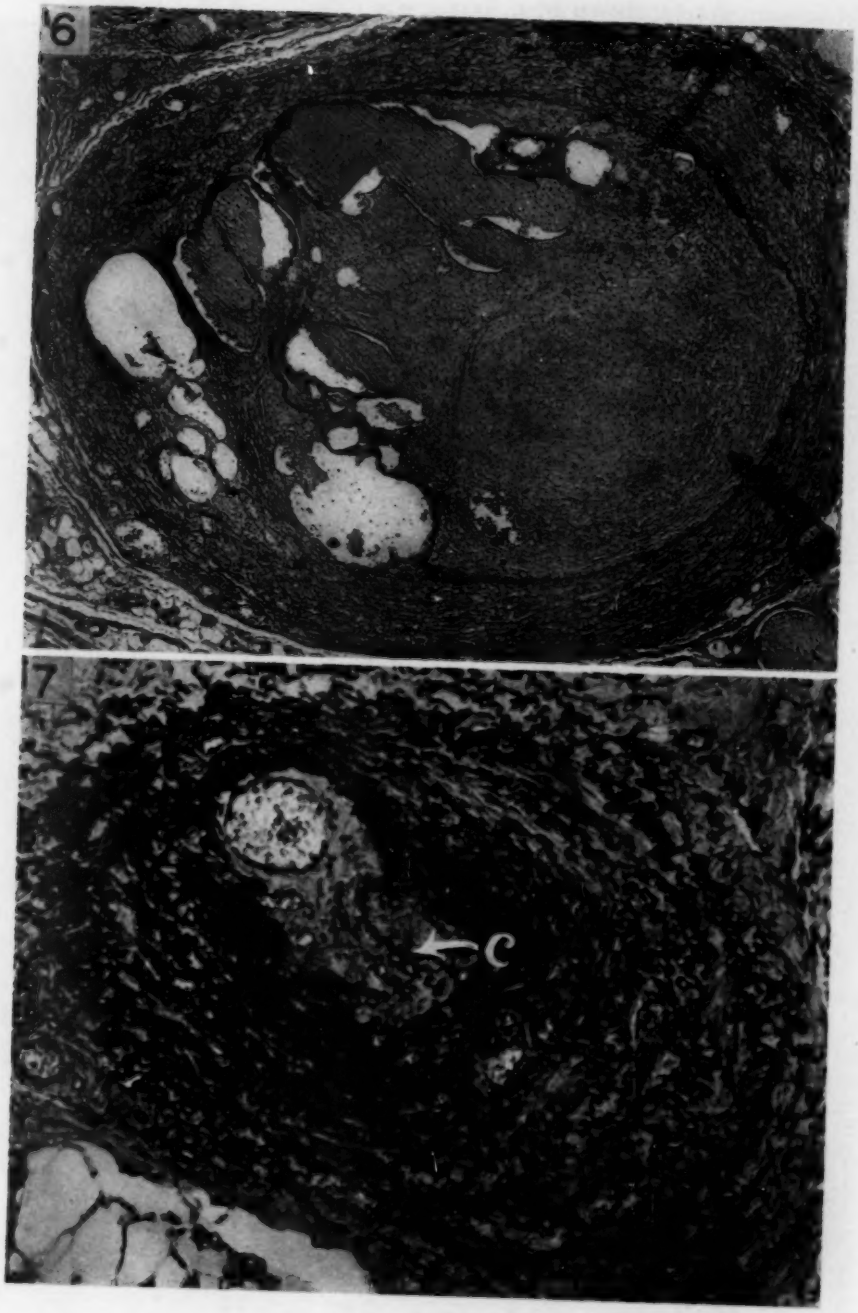
Explanation of Figures 2 to 5

Fig. 2.—The involvement of a valve in the granulomatous inflammation. Almost an entire cusp is incorporated in the tissue which fills the valve sinus. Phosphotungstic acid–hematoxylin stain. × 124.

Fig. 3.—The interadhesion of the cusps brought about by fibrous tissue. It represents the end stage of either the inflammatory process or the local thrombosis. The typical granuloma was present in other sections of this case. × 112.

Fig. 4.—Early incorporation of the valve cusps in a thrombus that is being recanalized, engrafted on an inflammation. × 37.

Fig. 5.—Portion of a cusp from the previous figure, showing its relation to the granuloma and the thrombus. × 233.



Figures 6 and 7

(See legends on opposite page)

CASE 3.—A man was first seen at the Massachusetts General Hospital at the age of 37. He had had migrating phlebitis of the left foot for four months. There were no pedal pulses on this side. The toes were cyanotic and cold. No calcification of the arteries was seen in the roentgenograms. The Wassermann test of the blood was negative, and the urine showed no sugar. He admitted smoking since the age of 12; at first he used 10 to 15 cigarets a day, but admitted smoking as much as 6 pipefuls of tobacco plus 20 cigarets a day at the time of admission. He was seen at short intervals at the hospital for ulceration of the toes of both feet, and had an amputation of the right great toe two years after his first admission, which was followed in the next year by gangrene of the foot necessitating a Gritti-Stokes amputation of the right leg.

One year later, when he was 41, this same procedure was carried out on the left side. Vessels from the amputated limb were used in this study. The disease progressed to ulceration and development of gangrene in the fingers of both hands. The radial arteries at first showed diminution in size and at his last admission, at the age of 46, he had lost several fingers and parts of the remaining digits. There was no pulsation in the vessels at the wrists or the elbows.

CASE 4.—A man was first seen in the Massachusetts General Hospital at the age of 24 for pain and swelling of the right foot. He had had claudication in the right calf for one year, and four weeks before admission he had symptoms suggesting arterial thrombosis of the leg; he presented gangrene of the toes. The Wassermann test of the blood was negative; the urine showed no sugar. The vessels did not appear calcified in the roentgenogram. He admitted smoking tobacco since the age of 12; he had smoked 20 cigarets a day for ten years but had increased this to 30 to 40 cigarets a day for the two years preceding admission.

A Gritti-Stokes amputation of the right leg was done; thrombi of varying ages were found in the arteries and veins of this limb. He suffered migrating phlebitis of the left thigh and the right stump during the convalescence. Two years later a guillotine amputation of the left leg was necessary because of gangrene of the toes and infection. Vessels from the amputated part were used in the present study. Reamputation at the level of the lower part of the thigh was later necessary. This was followed by intractable pain in the stump, which was finally treated by an intrathecal injection of alcohol. The amputation stump was amputated in turn because of slough. Later that year the patient had a cerebrovascular accident with paralysis of the right side of the face and the right upper limb. It was learned that he died suddenly the following year at the age of 28; his physician believed the death was caused by pulmonary embolism.

EXPLANATION OF FIGURES 6 AND 7, SHOWING THROMBOTIC CHANGES IN THE VENOUS VALVES

Fig. 6 (case 2).—Organization and early recanalization of a thrombus in a vein have almost completely destroyed one cusp and have seriously disrupted the second. The typical granuloma was not found in this case. × 38.

Fig. 7.—The elastica of a cusp, *C*, lies within an organized thrombus. The enormous proliferation of the elastica of the vessel wall is often characteristic of thromboangiitis obliterans. The typical granuloma was present in other sections. × 150.

THE VALVE CHANGES

The disease process involving the valves is found to be a part of that involving the blood vessels in general. In order, therefore, to elucidate the lesions of the valves, we shall have to discuss to some extent the general pathologic aspects of this disease.

Three types of lesions are found in the blood vessels and valves: (1) inflammation, (2) thrombosis and (3) dilatation. In any one patient, and indeed in any one section of a vascular bundle, all three changes may exist side by side in the different veins and arteries.[5]

Inflammation.—The inflammation in thromboangiitis obliterans is characterized by an involvement of the entire vessel wall and perivascular region. The exudate contains many macrophages and lymphocytes and ordinarily but few polymorphonuclear leukocytes. The intima is thickened by hypertrophy of the elastica interna and by the growth of muscle cells and, particularly in the veins, by a focal inflammation with projection into the lumen of a nodular collection of inflammatory exudate. As already mentioned, this intraluminal tissue may or may not be a typical granuloma containing multinucleated giant cells. Buerger [6] originally described this tissue as developing within an organizing thrombus, but it probably precedes the thrombosis, since it can be seen in the absence of any thrombus.

Buerger indeed held that the organization of thrombi, which are widespread in this disease, accounts for the thickening of the intima and the intraluminal projection of tissue of whatever variety. One must, of course, admit that particularly in the veins there may be areas where the intima is thickened as the result of the recanalization of an obstructive thrombus or the simple organization of a parietal thrombus. But we cannot agree with Buerger that the greater part of this tissue is of such origin. There is ample evidence, too extensive to be reviewed here, that a thickening of vascular intima by proliferation of its cells or by inflammatory exudation occurs as a reaction to a great variety of stimuli. On the other hand, it is difficult to conceive of such universal thrombosis as would be necessary to account for the initial thickening wherever it is encountered. One may presume that under such conditions the clinical symptoms would be infinitely more severe than those one now sees in the disease. Finally, this tissue differs histologically from that which arises by the organization of a thrombus. Blood pigment is not seen; the tissue is not vascular, and neither is there any great increase in vascularity of the vessel wall, which, contrariwise, is so striking in the presence of a visible thrombus in this disease.

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5. Except when noted, all the preparations shown were made with Verhoeff's elastic tissue stain overlaid by Van Gieson's stain. Mallory's aniline blue and phosphotungstic acid–hematoxylin stains were used in some cases.

6. Buerger, L.: The Circulatory Disturbances of the Extremities, Philadelphia, W. B. Saunders Company, 1924.

The valve cusp alone may be the site of inflammation with resultant thickening and with disorganization of its structure. More commonly, the cusp is free of primary changes but becomes involved in the inflammation of the adjacent venous wall (fig. 2). In this way the cusp may be anchored in the exudate, becoming grossly inefficient or completely useless.

Resolution of the inflammation probably never allows the valve to return to normal, even if the cusp has suffered no intrinsic damage. Varying degrees of fibrosis result, leading to adhesion of the cusps to the intima of the venous wall or to each other, and thus their normal excursion is interfered with (fig. 3).

Moreover, the inflammation is often associated with thrombosis of the involved segment (figs. 4 and 5), which, as will be seen in the following section brings its own serious damage to the valve.

Thrombosis.—Thrombosis is a second prominent finding in thromboangiitis obliterans. The organization of the thrombus is accompanied by an unusually prominent vascularization of the vessel wall. Yet the internal elastic lamina remains prominent, though perforated by the vessels passing from the vasa vasorum to the thrombus. Recanalization occurs as in thrombi of any origin.

These processes of organization and recanalization affect the valves in the same deleterious manner as we have demonstrated them to be affected in thrombosis from other causes. The cusp, lying within the thrombus, shows early disruption of its structure by virtue of its participation in the organization of the thrombus. The capillaries at its base proliferate and extend toward its free margin; its endothelium disappears, and fibroblasts course through its substance. As collagen is laid down in the organizing thrombus, the cusp is incorporated in the new tissue. From this time on, its identity can usually be distinguished only with the aid of stains for elastic tissue, which demonstrate its elastic layer. But the elastica itself early shows twisting, reduplication, and interruption by the invasion of capillaries continuous with those of the adjacent organizing thrombus.

As recanalization proceeds, the ever widening blood channels progressively break down the structure of the cusp. When recanalization is complete, the only recognizable elements of the cusp are bits of its elastic tissue incorporated in the walls of the newly formed channel or channels (figs. 4, 5, 6 and 7).

In places, a thrombus may be deposited along the venous wall, without completely filling the lumen. Such mural thrombi are apt to be formed in the valve sinuses, i. e., to the outer side of the cusps. Here again the cusp may become adherent to, or incorporated within, the organizing thrombus, to an extent depending on the degree of its contact with the thrombus (fig. 8).

Dilatation.—Many valves show changes identical with those we have demonstrated in the varicose saphenous vein. In illustrating this lesion in thromboangiitis obliterans, we have chosen examples only in the deep veins, avoiding the superficial veins which might well have been subject to spontaneous varicose change.

A vein tends to be elliptic in cross section, with the cusps of the valve stretched across the long axis.[7] The dilatation at first takes place in this axis and acts on the valve commissure, which is the site of attachment of the two cusps to the wall of the vein. Early there is a thinning

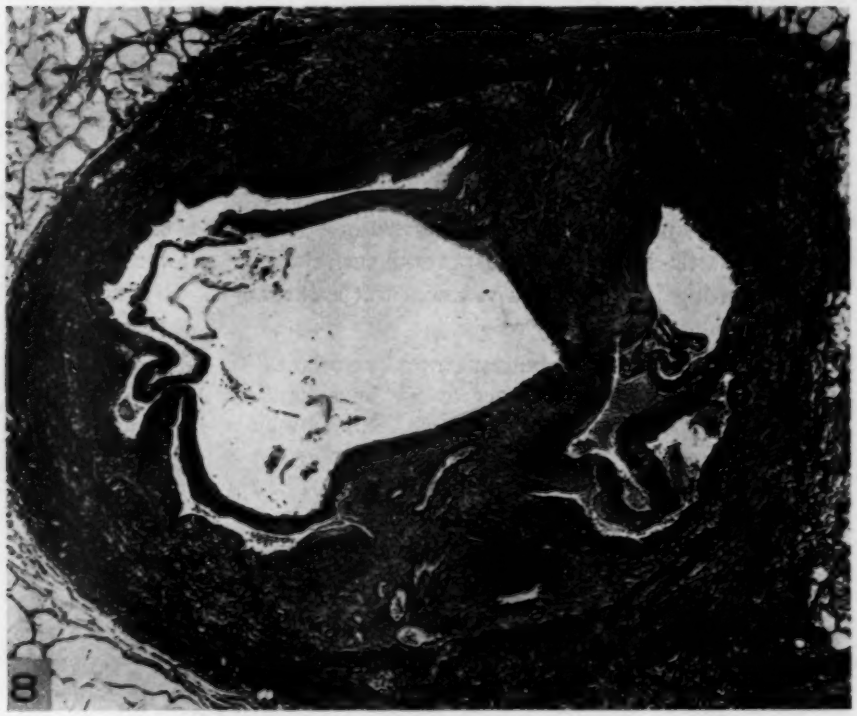
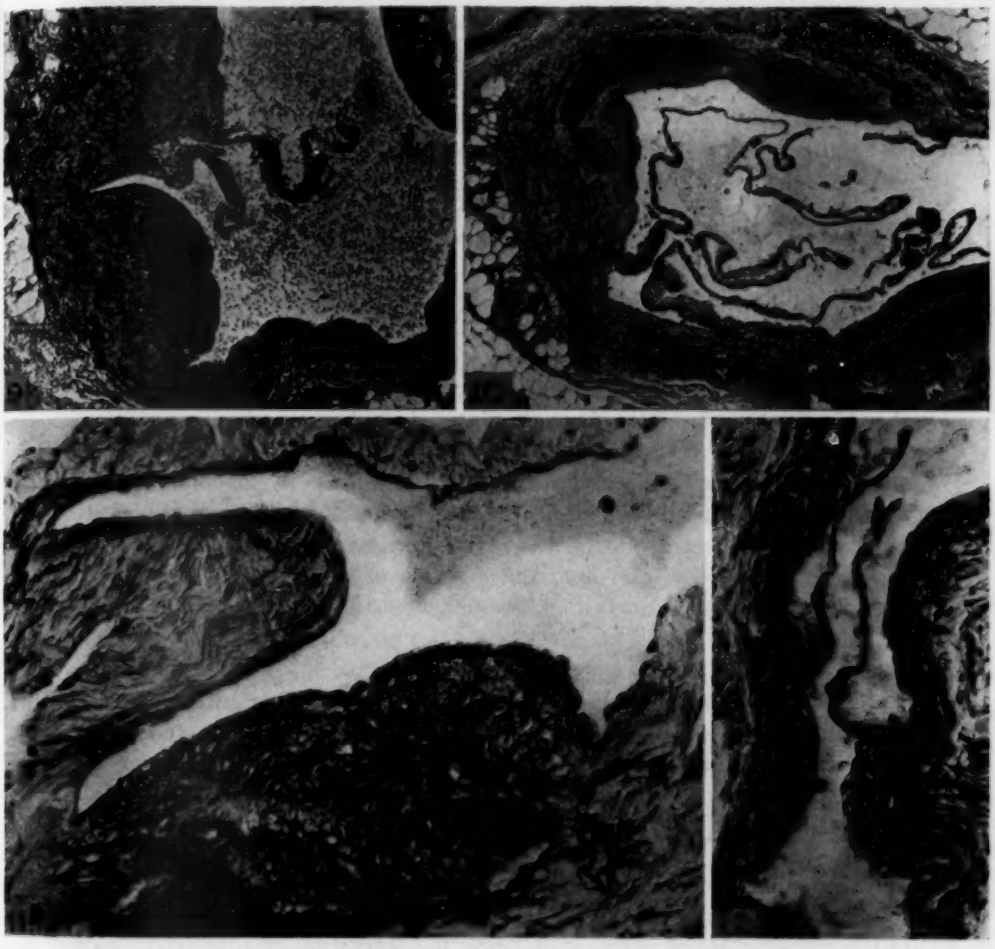


Fig. 8 (case 4).—Extensive adhesions of the valve cusps to an organized mural thrombus, with destruction of most of the valve. The typical granuloma was not found in this case. × 50.

of the mound of tissue normally present here, with the gradual development of a lateral cleft between the attachments of the cusps (fig. 9). As the dilatation proceeds, it may deepen the cleft into an evagination (fig. 10), and at about this time the vein widens in its shorter diameter. This increases the distance between the adjacent cusps. Both the evagination and the separation of the cusps thus result in incompetence of the valve; the first by producing a valveless channel lateral to the cusps, the second by preventing their approximation.

7. Edwards, E. A.: Anat. Rec. **64**:369, 1936.



EXPLANATION OF FIGURES 9 TO 12, SHOWING DILATATION CHANGES IN THE VENOUS VALVE

Fig. 9 (case 1).—The commissure of the valve shows disappearance of its usual mound of tissue. A niche has formed in its place, between the cusp attachments. The typical granuloma was not found in this case. × 41.

Fig. 10 (case 3).—Continued dilatation has widened the defect between the cusp attachments. The elastica connecting the cusps is thinned out. The typical granuloma was not found in this case. × 25.

Fig. 11.—The region of the commissure after extreme separation of the cusps. A mass of fibroblasts and muscle cells has grown into the defect. From the same patient as figure 3. × 235.

Fig. 12.—Longitudinal section of a valve, showing fibroblasts and muscle cells extending onto the contact surface of each cusp. From the same patient as figure 9. × 245.

Frequently these primary changes are associated with a reparative process which tends, though in vain, to fill in the anatomic gap caused by the processes of evagination and dilatation. This consists of a deposit in the defect of a mass of tissue composed of fibroblasts, large numbers of smooth muscle cells, elastic tissue and collagen (fig. 11). The tissue often grows along the contact surfaces of the cusps, resulting in further thickening and rigidity of the valve (fig. 12). Capillaries and blood pigment are characteristically absent. This, together with the presence of considerable smooth muscle, speaks very strongly against this tissue's having its origin in an organized thrombus.

We judge that the dilatation evident at the valve site is secondary to obstruction at proximal levels. This brings to mind the work of Thoma, who obtained intimal proliferation *on both sides* of experimental ligatures of both veins and arteries.[8] It was his thesis that slowing of the blood stream operated to produce this change whether or not dilatation was present. Areas of obstruction are of course widely distributed in the veins and arteries of the limbs in thromboangiitis obliterans. We may well inquire whether here as well the obstruction may not be responsible for much of the intimal proliferation found in the disease, of which the described reaction changes at the valve sites are but a part. We hope to inquire further into this matter.

SUMMARY

Examination of the blood vessels of the lower extremities shows that thromboangitis obliterans damages the venous valves extensively and seriously. The lesions involving the valves are part of the changes in the blood vessels in general—first, inflammation; second, thrombosis, and third, dilatation secondary to the obstruction by the inflammation or thrombosis.

The valves may be disrupted by the inflammation. Their excursion may be limited by their involvement in the inflammatory exudate in the valve or in the vessel wall, or by the formation of adhesions.

In obstructive thrombosis the valve is destroyed by the organization and recanalization, any remaining portions being incorporated in the walls of the channel or channels. In mural thrombosis the cusps may be incorporated in the organizing tissue or their excursion limited by thickening and adhesion.

The dilatation of the veins distal to areas of obstruction is associated with relative incompetence of the valve. The growth of reparative tissue often additionally thickens the cusps and makes them rigid. The thesis is presented that this may be but one part of a widespread proliferative change in thromboangiitis obliterans secondary to the obstruction of the blood vessels.

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8. Thoma, R.: Virchows Arch. f. path. Anat. **204**:1, 1911.

CHEMOTHERAPY OF EXPERIMENTAL STREPTOCOCCIC PERICARDITIS

A COMPARISON OF SULFANILAMIDE AND AN ACETYLATED DERIVATIVE OF 4,4′-DIAMINODIPHENYLSULFONE HYDROCHLORIDE IN THE TREATMENT OF EXPERIMENTAL BETA HEMOLYTIC STREPTOCOCCUS PERICARDITIS IN THE RABBIT

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Even though the cure of certain bacterial infections in man by members of the sulfonamide group of compounds represents a great advance in chemotherapy, the efficacy of these drugs is by no means ideal. Buttle,[1] Fourneau [2] and their co-workers reported a considerably higher degree of chemotherapeutic activity for 4,4′diaminodiphenylsulfone than for sulfanilamide in beta hemolytic streptococcus infections of mice. These reports were confirmed by other groups of investigators. Thus, Raiziss and his collaborators [3] have shown that this compound is ten times as effective as sulfanilamide in mice infected intraperitoneally with the C203 strain of the beta hemolytic streptococcus. The corresponding diacetyl derivative of this sulfone compound, although less toxic, is also less active. From these and other reports,[4] as well as from a thorough exploration of the literature in general, it appears that 4,4′-diaminodiphenylsulfone is probably the most therapeutically effective compound against the hemolytic streptococcus yet to be synthesized.

Unfortunately, the toxicity of this compound is so great as to forbid its use in both experimental and human streptococcic infections. Thus, in our preliminary trial experiments single daily doses of 0.3 Gm. of 4,4′-diaminodiphenylsulfone were administered by mouth for three to

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1. Buttle, G. A. H.; Stephenson, D.; Smith, S.; Dewing, T., and Foster, G. E.: Lancet **1**:1331, 1937.

2. Fourneau, E.; Tréfouël, J.; Nitti, F., and Bovet, D.: Compt. rend. Acad. d. sc. **204**:1763, 1937.

3. Raiziss, G. W.; Severac, M.; Moetsch, J. C., and Clemence, L. W.: Proc. Soc. Exper. Biol. & Med. **39**:339, 1938.

4. Bauer, H. S., and Rosenthal, S. M.: Pub. Health Rep. **53**:40, 1938. Feinstone, W. H.; Bliss, E. A.; Ott, E., and Long, P. H.: Bull. Johns Hopkins Hosp. **62**:565, 1938.

four days, starting twenty-four hours after the production of beta hemolytic streptococcus pericarditis in rabbits. Death was delayed only three to four days beyond the control period. Repeated attempts to increase the dose of the drug in the infected animals led to such severe acute toxic reactions that they were quickly abandoned.

Relatively few attempts [5] have been made to reduce the toxicity of this drug without excessive loss of activity by modification of its active amino groups through acetylation and through use of acyl substituents and Schiff bases. Although the derived products are less toxic, they are not as effective as the parent compound. Recently Roblin and co-workers [6] synthesized a new series of derivatives of 4,4′-diaminodiphenylsulfone, many of which are closely related to the original compound and two of which exhibited a high antistreptococcic action in mice.

In consideration of the high degree of antistreptococcic activity of 4,4′-diaminodiphenylsulfone, it was deemed desirable to diminish its toxicity without excessive reduction of its effectiveness. By preliminary treatment with 1 molar hydrochloric acid in vitro, it was converted into a hydrochloride with an increase in aqueous solubility; thus intestinal absorption would be promoted and more uniform and higher blood concentrations of its free and conjugated fractions would be assured. The coadministration of 1 molar acetic acid led to acetylation of its amino groups in vivo with consequent reduction of its toxicity.

The object of these experiments was to determine the comparative therapeutic efficacy of sulfanilamide and an acetylated derivative of 4,4′-diaminodiphenylsulfone hydrochloride in experimental beta hemolytic streptococcus pericarditis in the rabbit. Sulfanilamide was selected as the standard antistreptococcic agent among the present members of the sulfonamide group.

PREPARATION, PROPERTIES AND MICROCHEMICAL ANALYSES OF 4,4′-DIAMINODIPHENYLSULFONE AND THE ACETYLATED DERIVATIVE OF ITS HYDROCHLORIDE

The compound 4,4′-diaminodiphenylsulfone was originally prepared by Fromm and Wittmann [7] by a reduction of the corresponding dinitro compound that was obtained by oxidation of 4,4′-diaminodiphenylsulfide.

5. Buttle, G. A. H.; Dewing, T.; Foster, G. E.; Gray, W. H.; Smith, S., and Stephenson, D.: Biochem. J. **32**:1101, 1938.

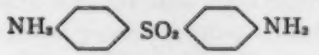
6. Roblin, R. O., Jr.; Williams, J. H., and Anderson, G. W.: J. Am. Chem. Soc. **63**:1930, 1941.

7. Fromm, E., and Wittmann, H.: Ber. d. deutsch. chem. Gesellsch. **41**:2264, 1908.

Raiziss and co-workers [8] have described several improved methods for its preparation, one of which consists in oxidation of 4,4′-diaminodiphenylsulfide (thioaniline) with protection of the amino groups by acetylation and subsequent hydrolysis. By this procedure a yield of 80 per cent of a fairly pure product was obtained, which was further purified by recrystallization from 50 per cent alcohol.

The preparation used in these experiments was prepared from pure 4-nitro-4′-aminodiphenylsulfide as a starting material by oxidation, and then recrystallized several times for purity.[9]

It is a crystalline solid compound, consisting of almost colorless long rectangular plates. It possesses the following structural formula:



It is sparingly soluble in water at room temperature, only to the extent of 0.01 per cent, and is but slightly more soluble in hot water, to the extent of 0.05 per cent. Its melting point is 176 C. Certain physical and chemical constants, found by microchemical determinations and run in duplicate, averaged as follows: carbon, 57.87 per cent; moisture content, 1.30 per cent; nitrogen, 11.00 per cent; hydrogen, 5.16 per cent; sulfur, 12.91 per cent. The samples left no ash. These determinations were carried out by Dr. Carl Tiedcke, Laboratory of Microchemistry, New York. The calculated percentages for nitrogen and sulfur were, respectively, 11.29 and 12.99. Thus, the found and the calculated percentages for nitrogen and sulfur were in good agreement, indicating that the prepared compound was of high purity.

The product was converted into a hydrochloride by the addition of 1.0 cc. of 1 molar hydrochloric acid to 0.1 Gm. of drug. Chemical analysis of the compound for the hydrochloride radical showed that the reaction was completed within thirty minutes.

DETOXIFICATION OF 4,4′-DIAMINODIPHENYLSULFONE

The term "detoxification" as employed in this investigation implies either total abolition or reduction of the toxicity of a substance as the result of certain changes which it undergoes in the animal body. Acetylation is concerned in the detoxification of amino groups and represents a mechanism by which the animal organism protects itself against foreign toxic substances.[10] A well known example of this is the acetylation of

8. Raiziss, G. W.; Clemence, L. W.; Severac, M., and Moetsch, J. C.: J. Am. Chem. Soc. **61**:2763, 1939.

9. The preparation was furnished by Dr. G. W. Raiziss, Dermatological Research Laboratories, Division of Abbott Laboratories, Philadelphia.

10. Sherwin, C. P.: Physiol. Rev. **2**:238, 1922. Stekol, J. A.: Ann. Rev. Biochem. **10**:265, 1941.

the amino group of p-aminobenzoic acid. Klein and Harris [11] found that the acetylation of sulfanilamide in vitro by slices of rabbit and human liver is determined by the rate of the production of acetate by the tissues. The addition of acetate or of its precursors in the tissues increases the degree of acetylation of sulfanilamide. Acetylation occurs only in the presence of intact and functioning liver cells and is not reversible. James [12] confirmed the observations of Klein and Harris and noted an increased excretion of acetylsulfanilamide in the urine of mice when sodium acetate was fed with sulfanilamide.

With the object of reducing the acute toxicity of 4,4′-diaminodiphenylsulfone, experiments were carried out with ascorbic acid, nicotinic acid, dextrose and acetic acid and its precursors in vivo, namely, lactic and pyruvic acids, and with cystine, glycine and other substances. After many trials, 1 molar acetic acid was selected as the most satisfactory agent for this purpose. It provides directly the acetyl radical which replaces in part or in full the amino groups. The administration of acetic acid with the sulfone compound definitely protects the rabbit against the acute and chronic toxic effects of the sulfone compound by lessening their frequency and severity. At the same time it does not materially affect the curative value of the drug. It appears that acetylation of the sulfone hydrochloride serves as a detoxicating mechanism. It is also of interest to observe in different infected animals that there is considerable variation in the acetylation of the drug even in the presence of added 1 molar acetic acid.

MATERIAL AND METHODS

Rabbits.—Throughout this series of experiments, normal adult male rabbits, 6 to 7 pounds (2.5 to 3 Kg.) in weight, of the breed known as the New Zealand white, were used. It was found that the weight, sex and breed were significant in that the period of survival of the untreated control animals and the incidence of toxic reactions in the treated groups were somewhat dependent on these factors, fluctuating especially with the weight. Each animal was maintained under constant dietary conditions and housed in the same individual cage for one week or more prior to operation. The animals proved to be uniformly susceptible to intrapericardial infection with the beta hemolytic streptococcus.

The Strain of the Beta Hemolytic Streptococcus and the Size of the Inoculum Introduced into the Pericardial Sac.—The strain of the beta hemolytic streptococcus originally isolated by Gay from the pleural cavity in a fatal case of human empyema was employed in this investigation. After being passed through the pleural cavities of many generations of animals, its pedigreed stock culture, designated H, has attained and retained a standard virulence for rabbits. The strain belongs to Lancefield's group A and is smooth both as to virulence and as to colony form.

11. Klein, J. R., and Harris, J. S.: J. Biol. Chem. **124**:613, 1938.

12. James, G. V.: Biochem. J. **33**:1688, 1939; **34**:633, 1940.

Its virulence was preserved by desiccation of the cultures and storage in the frozen-dried state with suppression of their metabolic processes:[13] From a blood agar plate culture, representative hemolytic colonies were inoculated into beef infusion broth and incubated at 37 C. for twenty-four hours. Subcultures in this broth were stored in the cold for twenty-four hours. Concentrated suspensions of bacterial cells from the broth cultures incubated for fifteen hours were then frozen and desiccated over phosphorus pentoxide. The tubes of dried cultures were sealed and stored in the icebox at 3 to 6 C. Their virulence was preserved in this fashion for at least six months.

Three days before the production of the suppurative pericarditis, 0.5 cc. of veal infusion broth was added by pipet to a tube of dried culture, and 0.2 cc. of this suspension was transferred to veal infusion broth. Transplants were made in this medium twice daily for three successive days. From a five hour subculture, a 4 mm. platinum loopful was suspended in 5.0 cc. of tryptose broth, and from this 1:10, 1:100 and 1:1,000 dilutions were made in tryptose broth. Duplicate plates were made with 0.12 cc. of each of the dilutions in 10 cc. of freshly melted tryptose agar, incubated at 37.5 C. for forty-eight hours and the colonies counted with a colony counter. Simultaneously with these platings, 0.12 cc. of each dilution was inoculated intrapericardially into a series of 4 rabbits to determine the lowest number of micro-organisms capable of producing a fatal infection within three to five days. This was generally found to be between 3,000 and 5,000 colonies per 0.12 cc., each colony representing a single bacterium or an aggregate of bacterial cells. This procedure was repeated with each group of controls and of treated animals. Within certain limits it was found that the size of the infective dose was not definitely related to the duration of the pericarditis; rather, the duration was a function of the breed and sex, and especially of the weight of the animals.

The Production of Experimental Beta Hemolytic Streptococcus Pericarditis in the Rabbit by an Extrapleural Route and in a Single Stage Operation.—Despite the delicacy of the pericardial membranes of the rabbit, the pericardial cavity can be exposed without entrance into either pleural sac, because of the widely separated and complete mediastinal partitions.

After the usual preparations of the anterior thoracic wall, the skin and subcutaneous tissue over the lower third of the sternum were incised in the midline under preliminary anesthesia induced with soluble pentobarbital followed by light inhalation of ether to induce satisfactory relaxation. The underlying fascia and muscles were cleared away from the ribs on both sides of the sternum. With a small bone-cutting forceps, the sternum was resected transversely between the seventh and eighth ribs, and then these ribs were divided as close as possible to the sternal margins to avoid injury to the internal mammary arteries. Their injury led to troublesome and even fatal hemorrhage. The mammary vessels are situated at some distance from the sternum and, if necessary, can usually be sufficiently retracted so that their ligature is unnecessary. Experience also showed that it was important not to sever the ribs at a point too distant from their sternal attachments; otherwise fatal pneumothorax might follow from injury to the pleural membranes. The approach described provides a satisfactory exposure of the pericardial sac.

After the removal of the resected piece of sternum, the beating heart was easily visible through the transparent pericardium. Any fat over the parietal layer

13. Wadsworth, A. B.: Standard Methods of the Division of Laboratories and Research of the New York State Department of Health, ed. 2, Baltimore, Williams & Wilkins Company, 1939, p. 57.

obscuring the view of the heart was carefully dissected away. The parietal pericardium was seized with a fine mouse-toothed forceps or hemostat, and the infecting dose of organisms, contained in 0.12 cc. of nutrient broth warmed to body temperature, was injected into the sac by means of a tuberculin syringe with an attached no. 26 hypodermic needle, with the usual precautions for asepsis. Care was taken not to injure the coronary vessels or the myocardium. The needle was then withdrawn, the puncture wound closing rapidly without apparent leakage of the inoculum into the adjacent tissues. If desired, the puncture wound of the pericardium may be securely ligated beneath the tip of a hemostat. Replacement of the resected piece of sternum was not necessary. The incision was sutured layer by layer. The wound was dressed with sterile gauze, and the animals recovered rapidly from the operation.

Another satisfactory approach to the pericardium is through a small opening on the left or the right side close to the sternum. The third rib is exposed, and after 1 to 2 cm. have been resected, the intercostal muscles and membranes between the lower border of the second and the upper border of the fourth rib are cleared away.

Under the perfected conditions of experimentation and without resorting to any elaborate operative technic, uniformly fatal suppurative pericarditis was reproduced with absolute regularity within a narrowly defined period.

Blood Cultures.—The blood cultures were taken immediately prior to the administration of the drugs and daily in the untreated group, the group treated with the sulfone compound and the group treated with sulfanilamide until recovery or death. From the marginal vein of an ear 1 cc. of blood was withdrawn with an accurately graduated tuberculin syringe, and tryptose broth dilutions of the blood were immediately plated in cooled Bacto blood agar base and incubated at 37.5 C. for at least seventy-two hours. The colony counts represented the number of single cocci and aggregations of beta hemolytic streptococci per cubic centimeter of circulating blood; they yielded an approximate comparison of the different groups of animals with respect to the bacteremia present at various stages.

Chemical Study of Blood Specimens.—Blood was obtained routinely two hours after the administration of sulfanilamide and after the administration of the sulfone compound with concomitant administration of 1 molar solutions of acetic and hydrochloric acid. The free and the combined sulfanilamide and the acetylated derivative of the 4,4′-diaminodiphenylsulfone hydrochloride in its free and in its conjugated state in the blood were determined by the method of Bratton and Marshall.[14] The sulfone compound yielded a purplish red reaction similar to that of sulfanilamide by diazotization and by coupling of the resulting diazo compound. The purplish red dye compound was estimated colorimetrically with the Klett-Summerson photoelectric cell colorimeter. The plasma carbon dioxide–combining power was estimated by the method of Van Slyke and the serum chlorides by the micromethod of Saiffer and Kornblum.[15]

Blood Counts.—Since the leukocytes of the blood of normal rabbits are subject to hourly variations, individual white cell counts were performed daily during the same period, namely, from 9 to 10 a. m., for several days preceding operation, to establish the general normal leukocyte trend for each animal. Two cover slip

14. Bratton, A. C., and Marshall, E. K. Jr.: J. Biol. Chem. **128**:537, 1939.

15. Saiffer, A., and Kornblum, M.: J. Biol. Chem. **36**:112, 1935.

blood films were prepared with Wright's stain at the same time, and 300 leukocytes were counted in each smear. The hemoglobin estimations were carried out by the Newcomer method.

The total number of leukocytes per cubic millimeter in the normal animals ranged from 5,200 to 14,300, with a mean average of 9,700. The average differential percentages were as follows: neutrophils (amphophils), 36; lymphocytes, 59; large mononuclear cells, 2; basophils, 2; eosinophils, 1. The average total red cell count was found to be 5,280,000 cells per cubic millimeter, and the hemoglobin content ranged around 85 per cent.

Histologic Studies.—Many representative blocks of myocardium and overlying visceral pericardium and of lungs, spleen, liver, kidney, bone marrow and brain were sectioned for microscopic study and stained with hematoxylin-erythrosin, Van Gieson's stain, Masson's trichrome *lichtgrün* stain, and the Gram-Weigert stain for bacteria. In addition, films of the pericardial exudate and sections of the inflamed pericardium were stained for fibrin by the method of Weigert and for oxidizing ferments by the method of Schultze.

ADMINISTRATION AND DOSAGE OF DRUGS

Altogether, 250 rabbits were infected intrapericardially and divided into five different groups, one of which served as a control. In the various series, treatment was commenced at four, twelve, twenty-four, and thirty-six hours after the injection of the micro-organisms into the pericardial sac. These groups were designated respectively as A, B, C and D.

Of importance for successful therapy are such factors as the virulence of the strain and the type of streptococcus, the lapse of time between infection and treatment, the maximum tolerated dose in grams per kilogram of body weight, the total dose of drug administered, the intervals between doses, the maintenance of an adequate and constant concentration of the drugs in the blood and tissues, the length of the period of therapy and the absence of severe untoward acute and chronic reactions. The dose of each drug employed in the various groups was computed on the basis of data relative to these factors obtained in preliminary trial experiments in normal and in infected rabbits.

Group A was treated with sulfanilamide, the administration of the drug starting four hours after the production of pericarditis. The drug was prepared in a 1 per cent aqueous solution, which was introduced directly into the stomach by means of a hard rubber catheter in amounts of 3.0 cc. every six hours during the life of the animal. The total daily dose was 1.2 Gm. and was within the limits of the maximum tolerated dose of 1.0 Gm. per kilogram found for the normal rabbit by Trefouël and co-workers.[16] The administration of the drug in aqueous solution rather than as a solid in gelatin capsules not only assured more constant absorption from the intestinal tract and more adequate concentration

16. Fourneau, E.; Tréfouël, J.; Nitti, F., and Bovet, D.: Bull. Acad. de méd. **118**:210, 1937.

in the blood, despite the relatively low aqueous solubility of the compound, but aided in providing an adequate fluid intake. The total blood sulfanilamide level fluctuated during treatment from 7.4 to 19.2 mg. per hundred cubic centimeters, of which 20 to 51 per cent was conjugated. It was observed that the conjugation of the drug varied greatly with different animals and that its rate and trend were characteristic for a given animal.

It is well known that the rabbit may manifest an irregular toxic response to sulfanilamide. However, this series exhibited no severe untoward symptoms with the dosage indicated in the foregoing paragraph. It should also be noted that the dose in terms of kilograms of body weight was far in excess of that ordinarily employed in human therapy and that treatment was commenced eight hours earlier than in the twelve hour group B, treated with the acetylated derivative of 4,4′-diaminodiphenylsulfone hydrochloride.

The schedule of dosage with the sulfone hydrochloride in the initial two critical days of infection was varied in the different groups. It was found that the less severely ill twelve hour interval group B tolerated the compound better than groups C and D. Thus, the dosage was adjusted to the severity of the pericardial infection and its resultant bilateral empyema and bacteremia. Group B received 2.4 Gm. in the first day and 2.0 Gm. in the second day in four divided doses, one being given every six hours. Groups C and D were given 2.0 Gm. each in the first day, and 1.5 Gm. and 1.0 Gm., respectively, in the second day of treatment in four divided doses at six hour intervals. A maintenance dose of 1.2 Gm. was given daily in three divided doses at eight hour intervals for ten successive days to each animal in groups B, C and D until recovery or death.

The drug suspended in 1 molar solution of hydrochloric acid was forced into the stomach through a hard rubber catheter, and acetic acid and physiologic solution of sodium chloride were then given immediately in the order named. The latter was discontinued after a few days, when the animals resumed eating and drinking. For each 0.1 Gm. of drug, 1.0 cc. each of 1 molar hydrochloric acid and 1 molar solution of acetic acid was given. Physiologic solution of sodium chloride was administered in 15 cc. amounts with the doses to maintain an adequate water-salt balance and to guard against the development of dehydration and urolithiasis.

Raiziss and co-workers[3] have shown that 0.3 to 0.5 Gm. of 4,4′-diaminodiphenylsulfone per kilogram was the maximum tolerated dose of normal rabbits. Single doses as high as 1.5 Gm. per kilogram, administered concomitantly with 1 molar solutions of hydrochloric and acetic acids, were readily tolerated by normal rabbits without acid or injurious drug effects. The doses of acetylated derivative of 4,4′-diamino-

diphenylsulfone hydrochloride in the infected animals were well within the single maximum tolerated dose for normal rabbits and, although relatively high, were well borne.

The drug was rapidly absorbed from the intestinal tract and was present in estimable amounts in the blood stream within half an hour after oral administration. Maximum concentrations were attained within two hours and maintained at peak levels for another two to three hours. Administration of the drug during the first two days of treatment led to a total blood sulfone concentration of 10 to 21 mg. per hundred cubic centimeters, of which 15 to 63 per cent was present as the active free amino fraction. Despite the simultaneous feeding of acetic acid providing an ample supply of acetyl radical, the rate and the degree of conjugation varied in different rabbits. However, conjugation was characteristically constant for the individual animal, in sharp contrast to the irregularities observed in the case of 4,4′-diaminodiphenylsulfone. The adequate and constant blood concentrations following the administration of the sulfone hydrochloride were attributed to the increased water solubility of the hydrochloride derivative, promoting more uniform intestinal absorption.

The therapeutic effects of the drug could not be consistently correlated with the total blood concentrations. However, there appeared to be a fairly consistent correlation between therapeutic effectiveness and the level of the free, active fraction. Thus, the most satisfactory results were obtained with a concentration of the free drug of between 4 and 8 mg. per hundred cubic centimeters.

The variations in the carbon dioxide capacity of the blood plasma were within or slightly below normal limits. In no instance did the carbon dioxide–combining power fall below 40 volumes per cent. The hydrogen ion concentration of the blood plasma remained at neutrality. Hyperventilation and other evidences of acidosis were not detected. The serum chlorides were normal.

TOXICITY

A group of 10 normal adult rabbits of the same breed employed in the experiments received the identical doses of acetylated derivative of 4,4′-diaminodiphenylsulfone hydrochloride at the same intervals and for the same period as the twelve hour set of treated animals. They were observed daily for four weeks for evidence of acute and chronic intoxication. None of the animals exhibited any untoward effects, such as cyanosis, dyspnea, anorexia, diarrhea or symptoms and signs referable to the nervous system.

Semiweekly examination of the peripheral blood revealed in the third week the development of early anemia, which progressed slightly, so that at the end of the period of observation the red cell count fell between 3,500,000 to 3,000,000 per cubic millimeter and the hemoglobin content between 65 and 57 per cent. These changes were accompanied by a rise

of the reticulocytes in the peripheral blood to 2 and 8 per cent. The leukocyte counts fluctuated within physiologic limits or were slightly raised above normal, but in no instance was there observed a condition comparable to agranulocytosis. Microscopic examination of the bone marrow after four weeks showed early inhibition of erythrogenesis. From their appearance, the animals were in good health. Thus, apart from a slight loss of weight and a progressive low grade anemia, this group showed no toxic reactions.

Despite the concomitant administration of 1 molar acetic acid, the treatment of all infected animals was attended by an incidence of 11.7 per cent toxic reactions. These were more frequent and more severe in the thirty-six hour set than in the others. The earliest toxic manifestation noted was cyanosis of the lips and nasal mucous membranes not associated with an increased respiratory rate. With progressive severity, variably severe dyspnea and muscular weakness of the limbs with stumbling gait and uncoordinated movements developed. However, these reactions were generally transient, lasting but one to two days and disappearing completely except in a small proportion of fatal cases.

Within two to three days after infection, the red cell counts and the hemoglobin values of the treated and the control group dropped sharply to 3,000,000 and 3,500,000 per cubic millimeter and 55 to 47 per cent. With recovery, regeneration of the red blood cells and hemoglobin, accompanied by increased numbers of reticulocytes in the peripheral blood, was rapid. In contrast, the red cell count and the hemoglobin value continued to fall sharply in the untreated group, reaching levels as low as 2,100,000 per cubic millimeter and 38 per cent shortly before death. The acute hemolytic anemia occurring in both sets is attributed essentially to the direct action of the beta hemolytic streptococci on the bone marrow and circulating erythrocytes rather than to the drug.

RESULTS OF TREATMENT AND COMMENT

An important requisite for therapeutic investigations on acutely infected animals is that the experimental infection should pursue a regular course, preferably with a fatal termination, in a narrowly defined period. This object was successfully attained by the constant death of all the control animals within three to five days after the production of the pericardial infection. Survival with cure of the suppurative pericarditis by resolution, by fibrosis or by both, prolongation of life, and death were adopted as the criteria for the assessment of the value of the therapy.

The most significant observation in this study was that of the comparatively high antistreptococcic activity and efficacy of the acetylated derivative of 4,4′-diaminodiphenylsulfone hydrochloride. Of the 50 animals comprising the twelve hour interval treated group B, there

are 49, or 98 per cent, cured survivors. The one death occurred on the twenty-third day of treatment. In group C there are 68 per cent cured survivors, with a prolongation of life of from eleven to twenty-two days (average, seventeen and eight-tenths days) in the remainder. The compound is clearly not very effective in the thirty-six hour interval treated group D, in which the cured survivors numbered only 10 per cent. The duration of life in this group is considerably decreased, ranging from six to sixteen days and averaging but nine and two-tenths days.

The results clearly show that the range of effective doses is not wide and that treatment to be most successful must be initiated at an early stage of the pericarditis, namely, twelve to twenty-four hours after the introduction of the organisms into the pericardial sac. Although the separate groups treated with the sulfone compound are not as large as those used by investigators of the effect of this compound on experimental streptococcic infections in mice, the results are significant in consideration of the absolute constancy of death within three to five days in the untreated control group.

It appears that the explanation of the high death rate in the severely ill group D is to be sought in failure of immune response and in depression of the activity of the host's phagocytic cells together with the relatively lower total dose administered in the critical first two days of the fulminating pericarditis. The individual variation in resistance is also a significant factor in modifying results despite the fact that the different groups of animals were of the same breed, weight and sex and were infected with approximately the same inoculum of the same culture under identical conditions of experimentation. Another factor responsible for the low percentage of survivors in this group may have been the presence of still undemonstrated substances in the pericardial exudate inhibiting the action of the sulfone compound.

Loewenthal [17] brought forward evidence to show that sulfanilamide and antistreptococcic serum, each acting in a different but complementary manner, exert a synergic and enhanced therapeutic action in mice infected with streptococci. A potent and homologous antistreptococcic serum and the sulfone compound investigated or one of proved superiority administered simultaneously might have acted in a similar manner with increased effectiveness in the severely ill animals of group D, leading to a higher rate of cures.

Figure 1 definitely shows that sulfanilamide failed to cure a single one of the animals treated and prolonged life for but one to seven days (average, three and eight-tenths days) beyond the control period despite the commencement of treatment only four hours after the production of the pericarditis and despite the adequate dosage of the drug. The failure

17. Loewenthal, H.: Lancet **1**:197, 1939.

of sulfanilamide to influence decisively the natural course of the pericarditis may be attributed to its lower chemotherapeutic activity in comparison with the sulfone compound, the severity of the infection and, perhaps, to inhibition by unknown substances [18] in the early stage of the pericarditis and union with proteins [19] leading to inactivation. It may be deduced that sulfanilamide is without marked beneficial action on experimental beta hemolytic streptococcus pericarditis in the rabbit.

The first signs of recovery in animals treated with the sulfone compound are a return of appetite with a resumption of eating and an increased intake of fluid on the first to third day after the commencement of treatment, accompanied by a rapid fall in temperature. The temperature of an acutely infected animal is apparently less susceptible to

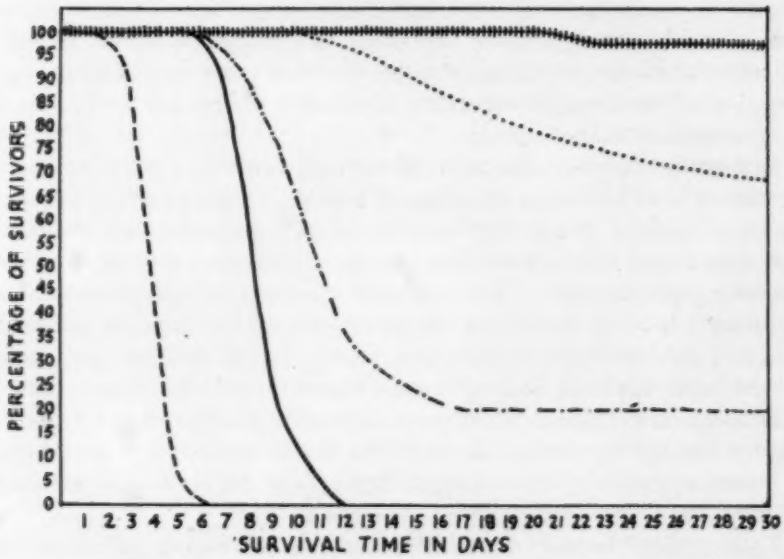


Fig. 1.—Effects of sulfanilamide and acetylated 4,4'-diaminodiphenylsulfone hydrochloride in experimental beta hemolytic streptococcus pericarditis in rabbits. The percentage of survivors in each group of 50 animals is shown by a curve: — — — —, control untreated group; ————, sulfanilamide-treated group, in which treatment was started four hours after the production of pericarditis; ++++, group in which treatment with the sulfone compound was started twelve hours after the production of pericarditis; ....., group in which treatment with the sulfone compound was started twenty-four hours after the production of pericarditis; . — . — . —, group in which treatment with the sulfone compound was started thirty-six hours after the production of pericarditis.

environmental influences than that of a normal one. Therefore, a fall is significant when correlated with other evidence.

18. Marshall, E. K., Jr.: Ann. Rev. Physiol. **3**:652, 1941.

19. Davis, B. D.: Science **95**:78, 1942.

In the absence of extensive streptococcic pneumonia the respiratory excursions do not rise above the normal rate of 30 per minute. In the animals that recovered the blood stream was rapidly cleared of circulating micro-organisms by 1.5 to 2.0 Gm. or more of the sulfone compound, as a rule, within twenty-four to forty-eight hours after the initial demonstration of the bacteremia. The rapid loss of body weight during the period of active infection is principally dependent on the local and systemic effects of the micro-organisms and, in a lesser measure, on the refusal of the animals to take food. Once recovery sets in, the gain in weight is rapid. It is of interest to note that animals dying two weeks or later after the commencement of treatment, despite an adequate daily maintenance dose, reveal no conspicuous symptoms.

Under the conditions of experimentation the curative effect of the sulfone compound is complete in the sense that the micro-organisms are not recoverable by culture from the tissues of the cured rabbits and that the pericarditis is completely healed by resolution, by fibrosis or by both.

The therapeutic efficacy of the acetylated derivative of the 4,4′-diaminodiphenylsulfone hydrochloride is apparently related to the presence of its two active paraamino groups, which are evidently made available by deacetylation in vivo in body fluids and tissues by reduction through hydrolysis of the acetylated derivative. The therapeutic effectiveness is governed also by the following factors: (1) the rapidity of absorption of the sulfone hydrochloride from the intestinal tract, evidently dependent on its solubility in water and lipids or on other mechanisms; (2) the speed of development and constant maintenance of an adequate and effective concentration of the agent in the blood with a suitable ratio of the free, unconjugated and acetylated fractions of the drug; (3) the rate and the degree of diffusion into the body tissues and fluids; (4) the elimination of the drug; (5) its toxicity to the host tissues and organs, and (6) its concentration within the bacterial cells, which is probably of the same order as that of the milieu. Most of these factors have already been discussed in other sections.

It is not certain that sulfur is a necessary element in a bacterial chemotherapeutic agent. Sulfanilamide contains sulfur in the form of the sulfonamide group (SO_2NH_2). Similarly, the acetylated derivative of 4,4′-diaminodiphenylsulfone hydrochloride contains sulfur, but whether or not the sulfur atom is indispensable for its therapeutic properties remains to be established. Nevertheless, the fact remains that no compound therapeutically effective in streptococcic infections has yet been synthesized which does not contain sulfur in some form.

The precise mode of action of the sulfone compound remains a problem for future investigation. It is apparently bacteriostatic in vivo and perhaps even bactericidal to a certain extent.

Ultimate recovery is determined by the inhibition of multiplication of the streptococci through the bacteriostatic action of the sulfone com-

pound aided by the phagocytic activity of the mobilized neutrophils and mononuclears. There is no proof that the drug stimulates the production of phagocytes and antibodies or promotes phagocytosis. The neutrophil and macrophage reaction is a constant accompaniment of recovery.

Microscopic observations on the animals put to death clearly show that the mobilization of the mononuclears in the visceral and parietal pericardium so significant in healing appears to be the result of proliferation of the local tissue histiocytes. Histologic examination affords no support for the assumption that the cells of the reticuloendothelial system cooperate in controlling the infections.

Are these results of significance for the treatment of hemolytic streptococcus infection in man? Since the dose employed in the rabbit multiplied weight for weight represents an enormous dose of the acetylated derivative of 4,4′-diaminodiphenylsulfone hydrochloride for man, the question naturally arises as to whether any inferences at all may be drawn from these experiments in respect to the use of the compound against human infections. It should, however, be noted that the experimental pericarditis in the rabbit is far more severe than spontaneous suppurative pericarditis in man. All untreated control animals show a mortality rate of 100 per cent, with death occurring within three to five days, whereas the death rate for suppurative pericarditis in man is stated to be between 30 and 50 per cent [20] and the process is of longer duration. In point of fact, pericarditis in the rabbit is rather comparable to that in the rare patients who from the outset are overwhelmed by the invading streptococci.

Another great difficulty lies in the transfer of results from an animal species to man, since different host species may vary either qualitatively or quantitatively in their behavior both to the pathogenic micro-organisms and to the drugs. Nevertheless, despite these limitations, the essential basis for the clinical use of new synthetic drugs must continue to be in their performance under experimental conditions.

PATHOLOGIC OBSERVATIONS ON THE EVOLUTION OF EXPERIMENTAL PERICARDITIS IN THE UNTREATED ANIMALS AND ON THE INFLUENCE OF SULFANILAMIDE AND ACETYLATED 4,4′-DIAMINODIPHENYLSULFONE HYDROCHLORIDE ON ITS COURSE

Controls and three sets of animals treated with the sulfone compound twelve and twenty-four hours and with sulfanilamide four hours after the inception of the infection were put to death serially in pairs at intervals of twelve, twenty-four and forty-eight hours and four, seven, fourteen and

20. Shipley, A. M., and Winslow, N.: Arch. Surg. **31**:375, 1935. Billings, A. E.: S. Clin. North America **12**:1517, 1932.

twenty-one days beyond the period in which death naturally occurred in the untreated animals. A study of the changes in the pericardial sac and other important organs was undertaken.

Evolution of the Pericarditis and Associated Lesions in the Control Animals.—Twelve hours after infection, changes in the pericardial sac are already discernible. The pericardial membranes appear dull and are covered with a delicate film of grayish exudate. Blood agar plates yield about five times as many single and chained cocci as were originally injected into the sac. The pleural cavities are still macroscopically normal and culturally sterile. The lungs are free of inflammatory consolidation. The other viscera are not remarkable on gross inspection.

At the twenty-four hour stage the pericardial sac contains 1.0 to 1.5 cc. of thin, yellowish white fibrinopurulent exudate. The pleural cavities and the lungs are usually still free of infection at this time.

At forty-eight hours and onward to the time of death the amount of fibrinopurulent exudate in the pericardial sac increases rapidly and markedly, so that at death, three to five days from the inception of the pericarditis, the sac is greatly distended by 6 to 10 cc. of thick, creamy, greenish or brownish gray fibrinopurulent effusion as measured with a syringe after agitation with a few cubic centimeters of sterile physiologic solution of sodium chloride. The pericardium is covered with opaque, grayish white, friable exudate from 1 to 3 mm. in depth, which strips without difficulty (fig. 2 *A*). Serofibrinous pleural infusion is constantly present at the forty-eight hour stage, later becoming frankly purulent and bilateral, and associated with multiple areas of red to grayish red hepatization in the lung. The empyema following the pericarditis does not appear to involve directly the parenchyma of the lung.

At death the lymph nodes draining the pericardium and situated at the base of the heart appear swollen and moist. The myocardium is the seat of diffuse severe parenchymatous degeneration. The cardiac valves are free of vegetations. There are widespread edema and suppuration of the thoracic wall, associated with extensive formation of tissue sloughs related to the puncture of the pericardial sac and injection of the infecting dose. The peritoneal cavity is not invaded by bacteria from either the pericardial or the pleural cavities. The spleen appears enlarged, friable and dark red, with extensive and marked obliteration of its normal landmarks. The stomach is acutely dilated. The liver and the kidneys show severe parenchymatous degeneration.

The pericardium of the normal rabbit contains but few mononuclear cells. It is externally lined with a single layer of flat serosal cells. Microscopic examination of the pericardium at the twelve hour stage of infection reveals universal vascular congestion and edema of the subserous layers associated with slight deposition of fibrin and formation

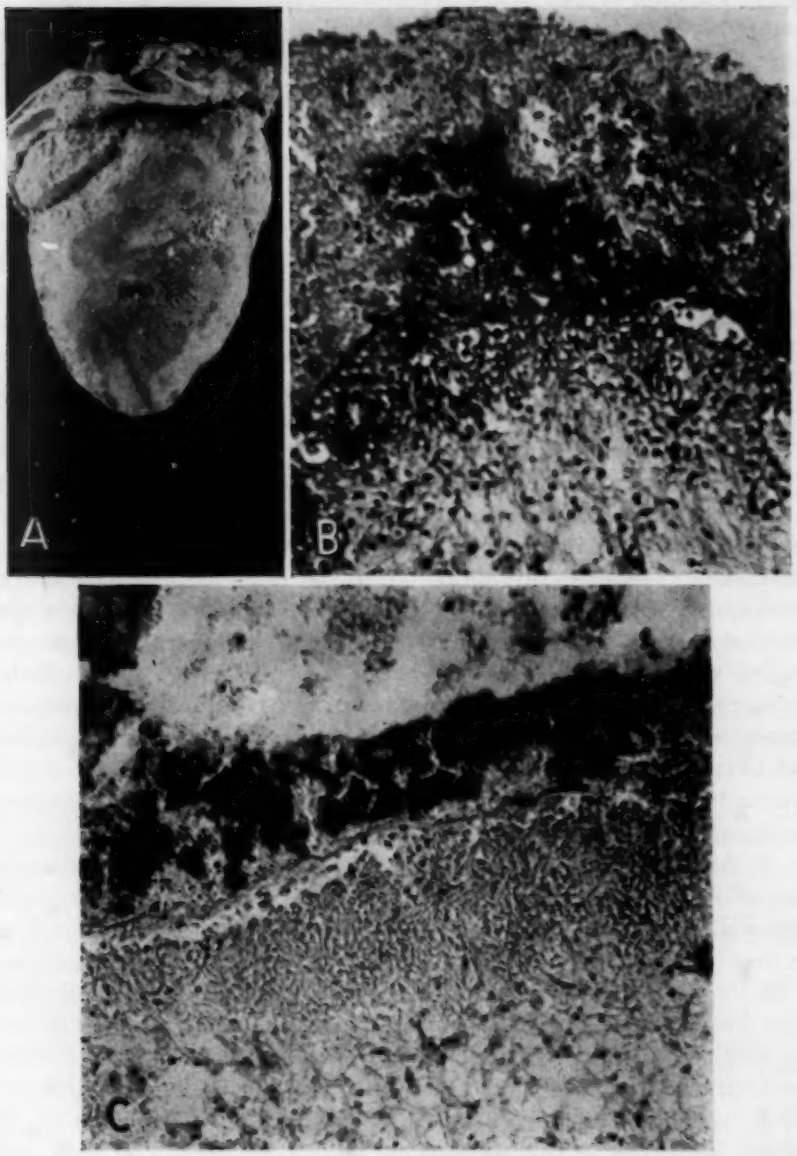


Fig. 2.—*A*, anterior view of the heart of an untreated rabbit which died on the fourth day, showing exudate over the visceral pericardium.

B, photomicrograph of a section of the heart shown in *A*, exhibiting heavy necrotic fibrinopurulent exudate over the visceral pericardium and edema and degeneration of the superficial myocardium; hematoxylin and erythrosin; × 210.

C, Photomicrograph of a section of the heart of a sulfanilamide-treated rabbit dying seven days after the production of the pericarditis. Note the myriads of gram-positive streptococci in the visceral pericardium and the characteristic degeneration and necrosis of the cellular exudate; Gram-Weigert stain for bacteria; × 230.

of cellular foci consisting almost entirely of neutrophils as observed in the oxidase-stained preparations. At this stage sections stained for bacteria by the Gram-Weigert method show but a relatively small number of cocci, for the most part extracellular. In the successive series there is an increasingly heavy deposit of fibrin and leukocytes with the neutrophil as the dominant cell type. This is accompanied by interstitial edema and degeneration and necrosis of the superficial muscle fibers of the myocardium. The leukocytic exudate (fig. 2*B*) from the second day onward is distinguished by progressive degeneration and necrosis attended by steady increase in the number of cocci. Myriads of streptococci are present at the time of natural death, but they are very largely extracellular and gram-positive in their staining reaction. Phagocytosis is not common, the ingested cocci appearing, as a rule, involuted and, at times, in short chains. No attempt was made to stain the capsules of the streptococci in the tissues.

Evolution of the Pericarditis and Associated Lesions in Rabbits Treated with Sulfanilamide.—In degree and extent the pericarditis and the local lesions accompanying it are indistinguishable on macroscopic examination from those observed in the untreated pairs of animals. Histologically, the pericardial exudate differs from that in the controls by a slight shift in its cellular composition toward the mononuclears, which still constitute the minority group of cells, and by a slight increase in phagocytosis. The micro-organisms are unlimited in number at death and are chiefly extracellular (fig. 2*C*).

Evolution of the Pericarditis and Associated Lesions in Rabbits Treated with the Acetylated Derivative of 4,4'-Diaminodiphenylsulfone Hydrochloride.—A comparison of the suppurative pericarditis and its local and distant complications in the sulfone-treated, the sulfanilamide-treated and the control groups clearly reveals that the sulfone compound not only profoundly alters the course and spread of the pericarditis but the histologic pattern of the infection as well.

These changes are already evident in rabbits of the twelve hour treated group put to death after twenty-four hours of treatment. Postmortem examination reveals no gross evidence of fluid exudate in the pericardial sac except for a few drops of dark blood-stained sterile fluid. The pericardium is restored to its normal state except for a few small irregular opaque adherent areas of grayish white exudate undergoing organization. There is no evidence of spread of the pericardial infection into the mediastinum or into the pleural cavities. The lungs are free of consolidation. The intestines are not distended. The liver, the spleen and the kidneys are extraordinarily free of pathologic changes. Both the kidneys and the bladder show no deposition of crystals or formation of concrements at this stage or later. The essential healing process in

this group of animals as revealed in the successive stages is that of resolution with or without fibrosis (fig. 3).

The twenty-four hour interval pairs of animals put to death after one day of treatment show from 0.7 to 1.0 cc. of thin straw-colored fluid effusion within the pericardial sac associated with multiple irregular deposits of exudate in an early stage of organization and a few small gray to pinkish yellow semigelatinous masses loosely adherent to the visceral and the parietal pericardium. Frank purulent exudate is absent. Macroscopic examination of the lungs reveals no inflammatory consolidation. The animals put to death after four days contain no measurable fluid exudate within their pericardial sacs.

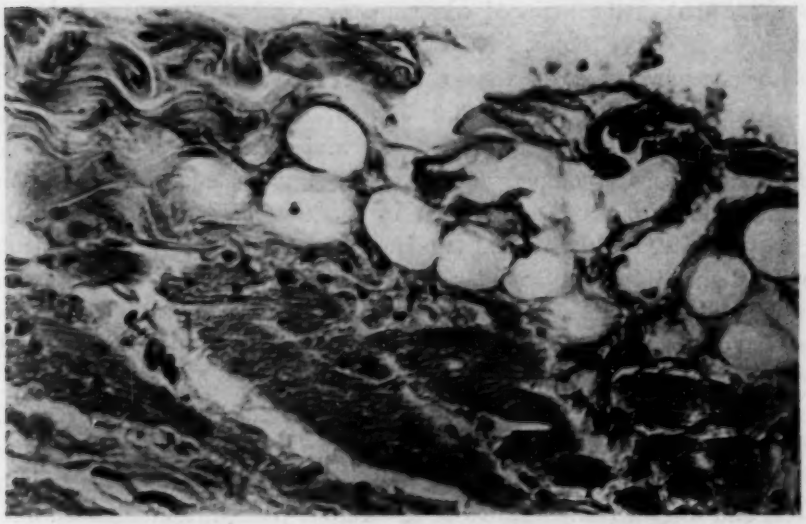


Fig. 3.—Photomicrograph of a section of the heart of a rabbit on the twenty-first day after a twelve day course of treatment with acetylated 4,4′-diaminodiphenylsulfone hydrochloride commenced twelve hours after induction of the pericarditis. Note healing by resolution and by fibrosis. Hematoxylin and erythrosin; × 380.

In comparison with the pericardial exudate in the control and the sulfanilamide-treated animals, that in the twelve hour and the twenty-four hour interval sulfone-treated animals presents a distinct change in its cellular composition as a result of treatment. After twenty-four hours of treatment the neutrophils are present in large numbers, distinctly predominating over the macrophages. Both (fig. 4 *A*), especially the macrophages, are actively phagocytic for bacteria, which are strikingly reduced and chiefly intracellular. There is a rough parallelism between the intensity of the mobilization of the neutrophils and mono-

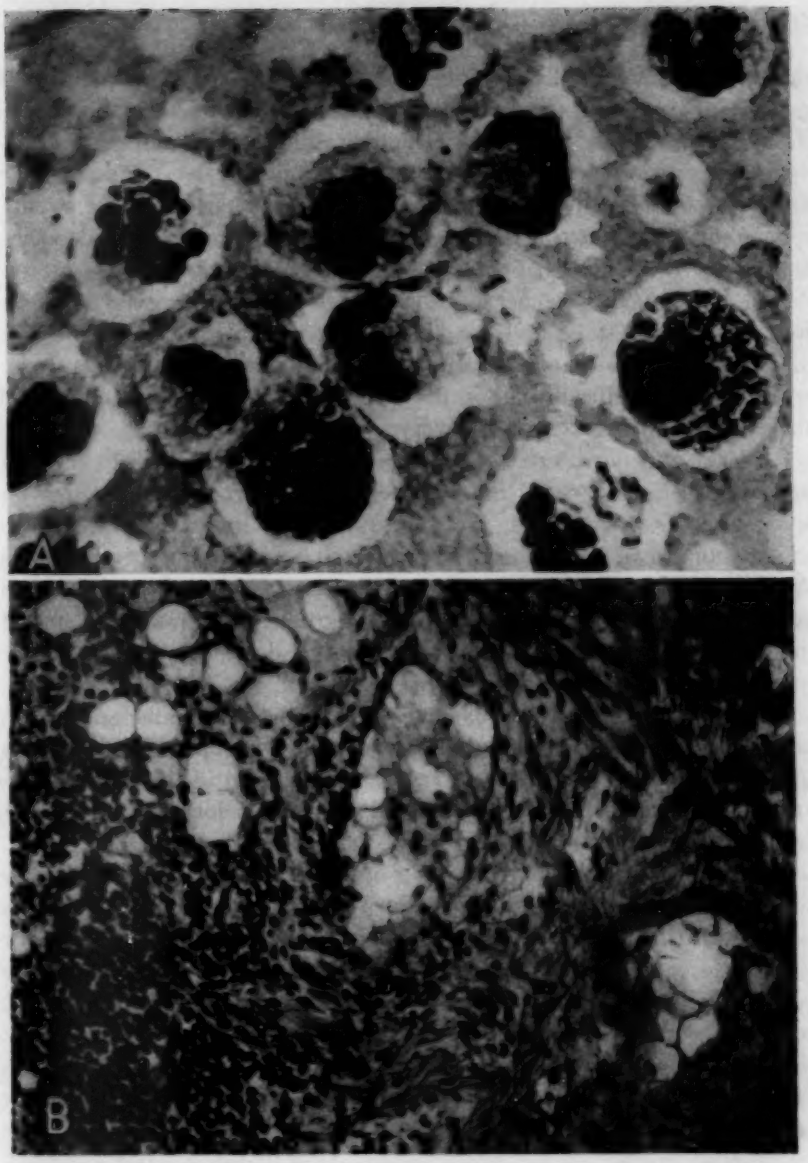


Fig. 4.—*A*, oil immersion view of a film of pericardial fibrinopurulent exudate from a rabbit after a twenty-four hour course of treatment with acetylated 4,4′-diaminodiphenylsulfone hydrochloride started twelve hours after production of the pericarditis. Note the paucity of extracellular organisms and the phagocytosis of bacteria by neutrophils and macrophages, especially the latter. Gram stain; × 1,600.

B, photomicrograph of a section of the heart of a rabbit after a seven day course of treatment with acetylated 4,4′-diaminodiphenylsulfone hydrochloride started twenty-four hours after the production of pericarditis. Observe the heavy infiltration of mononuclear cells associated with the deposition of fibrous tissue about remains of adipose tissue of the pericardium. Gram-Weigert staining for bacteria in sections from corresponding blocks reveals absence of bacteria. Hematoxylin and erythrosin; × 260.

nuclear cells and the disappearance of the organisms. At this stage the neutrophils play the principal role in the disposal of the micro-organisms. The cellular exudate in the twelve hour group thereafter undergoes rapid degeneration and necrosis, disappearing completely by the fourth day after the commencement of treatment. On the other hand, the twenty-four hour group shows a progressive increase in number and phagocytic activity of the macrophages at the forty-eight hour stage and at four days. These cells become the dominating ones in the exudate at the forty-eight hour stage and are chiefly responsible for the phagocytosis of any surviving organisms. It is clear that phagocytosis plays a conspicuous and significant part in the disposal of the micro-organisms and, therefore, in the process of recovery.

Edema and fibrin are not abundant in the pericardial exudate, and necrosis of the superficial muscle fibers of the myocardium is not conspicuous. Associated with the appearance and increase in number of the mononuclear cells, there is progressive dissolution of the fibrin. At first focally distributed in the areolar tissue beneath the epicardial serosa, the mononuclear cells increase progressively in numbers, spreading diffusely over the visceral pericardium and down the fibrous septums into the superficial layers of the myocardium.

Microscopic evidences of pleuritis or of pulmonary inflammation are not observed at any stage in the sulfone-treated groups. Histologically, the kidneys reveal no deposit of crystalline material in the renal tubules and pelvic cavities nor was there evidence of "pyelonephritis" possibly resulting from irritation by crystals of the drug.

Healing in the twenty-four hour interval animals treated with the sulfone compound takes place chiefly by organization with granulation tissue. On the fourth day after the initiation of treatment the reparative phase of the healing process, marked by abundant proliferation of young fibroblasts and capillaries, overlaps the exudation of mononuclear cells (fig. 4 *B*). Accompanying the disappearance of the mononuclears there is progressive deposition of fibrous tissue, ultimately leading to fibrous union of the two pericardial layers and bacterial sterilization of the sac.

RELATIONSHIP BETWEEN WHITE BLOOD CELL COUNT, BACTEREMIA AND MORTALITY

Early and initially in the control animals there is leukopenia due very largely to the reduction in number of the neutrophils. This leukopenia persists until death, becoming maximum at twenty-four to ninety-six hours after infection, with the number of white cells ranging between 3,000 and 4,500 per cubic millimeter.

In the twelve hour group of rabbits treated with the sulfone compound leukocytosis (leukocyte counts from 15,000 to 30,000) is nearly

always present at twenty-four to forty-eight hours after the institution of treatment. At times rising to higher levels, it is maintained for another two to five days, the number of white cells declining rapidly to normal thereafter.

In the twenty-four hour sulfone-treated group the initial leukopenia generally continues for another day or so after the onset of the pericarditis. The total white cell count then rapidly rises to 20,000 or more in the large majority of recovered animals. On the other hand, most of the animals succumbing to the infection show a progressive reduction in the white cell count to levels of 4,500 or lower after temporary leukocytosis.

The thirty-six hour treated group reveals early profound depression of the white cell count below normal. Except for infrequent brief rises to normal or above, the depressed white cell count persists in 86 per cent of the fatal infections.

The number of circulating leukocytes and the fluctuation thereof constitute a significant prognostic but by no means infallible measure of the probable course of the infection and of its response to treatment. The white cell counts at the twenty-four hour and forty-eight hour stages of infection prove to be more significant indicators of the outcome of the suppurative pericarditis at this time than the bacteremia. The fatality rate in all the sulfone-treated animals with counts of 20,000 or more after one to two days of treatment in the groups whose treatment was started after twenty-four hours is low.

There is a striking relationship between the time of appearance and the degree of bacteremia and the level of the white blood cells. The number of leukocytes is roughly inversely proportional to the frequency of bacteremia, which occurs least often with persistent white cell counts of more than 20,000. In general, the earlier and the more profound the depression of the white cell count, the higher is the incidence of bacteremia and the higher the colony count of streptococci per cubic centimeter of blood. Thus, in groups C and D, rabbits showing persistent counts of 4,000 or less, the blood cultures showed 1,000 or more colonies of streptococci per cubic centimeter of blood, with a resultant mortality of nearly 100 per cent. With disappearance of the organisms from the circulating blood as the result of treatment, the white cell count rapidly rises twenty-four to forty-eight hours later to normal limits or higher. The development of continued and progressive bacteremia in the face of treatment is indicative of impending death and provides more significant information as to the outcome of the infection at the forty-eight hour and seventy-two hour stage and thereafter than the total white cell count.

A distinct correlation exists between the time of onset and the severity of the bacteremia and the death rate. Thus, the earlier and

the more severe the bacteremia, the higher is the fatality rate. Progressive bacteremia with 1,000 colonies or more per cubic centimeter of blood, resistant to treatment, invariably proves fatal. In the control group the organisms may be detected in the circulating blood as early as twelve hours and always at forty-eight hours after the inception of the infection. The aggregation of streptococci at the twelve hour stage averages from 50 to 200 per cubic centimeter of blood. The number of circulating organisms increases in a strictly geometric progression, so that shortly before death they number 100,000 per cubic centimeter of blood or more. This number appears to constitute the lethal level.

SUMMARY

It has been shown for the strain of beta hemolytic streptococcus investigated that an acetylated derivative of 4,4′-diaminodiphenylsulfone hydrochloride exercises a distinctly beneficial effect on the natural course of experimental beta hemolytic streptococcus pericarditis in rabbits. It considerably increases the number of cured survivors in comparison with their complete absence in the sulfanilamide-treated group, and definitely prolongs the average duration of life beyond that of the control and that of the sulfanilamide-treated group. The most effective results were obtained in a group of 50 rabbits whose treatment was initiated twelve hours after the production of the pericarditis; 49 of these animals were cured. The incidence of survivors and the prolongation of life are definitely related to the time of starting treatment.

The toxicity of this compound is of a relatively low order.

The results are on a scale sufficiently large to indicate that the acetylated derivative of 4,4′-diaminodiphenylsulfone hydrochloride is far superior to sulfanilamide in its therapeutic effect on beta hemolytic streptococcus infection of the pericardium as observed in rabbits.

STUDIES OF CARTILAGE

IV. A MORPHOLOGIC AND CHEMICAL ANALYSIS OF AGING HUMAN COSTAL CARTILAGE

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There is a belief that at least three systems govern the deposition of calcium in human tissues. One is concerned with the regulation of special ionic equilibriums in circulating bodily fluids. The second is concerned with particular activities of cells in those regions which undergo calcification. The third is the matrix in which calcium is deposited. No theory of calcification comprehends the means by which the three systems operate. The importance of the regulation of special ionic concentrations in the blood has been established, but the basic determinants of calcification lie within the sphere of composition and function of specialized cells and matrices. One of the simplest examples of the participation of the second and third systems is the gradual deposition of calcium in aging costal cartilage. In this report the sequences in this process are described histologically and by quantitative analyses for lipids, iron, calcium and chondroitin-sulfuric acid.

METHODS

Preparation of Cartilage.—Tissue was obtained post mortem from 21 persons, aged 2 to 73 years. The third to sixth costal cartilages were resected and the perichondrium scraped away from the underlying matrix. The cartilages, free from perichondrium and other adherent tissues, were then divided transversely into blocks measuring 8 mm. in length. Several blocks of uniform appearance were selected from the group. If calcium deposits were recognized, only those blocks with similar axial deposits were retained for analysis. All blocks containing massive deposits of calcium and bone, especially in subperichondrial areas, were discarded.

The selected blocks, usually 3 to 5, were divided transversely into two parts, one part measuring about 3 mm. in length and the other about 5 mm. The smaller segments were prepared in permanent sections for microscopic study. The larger blocks, while still fresh, were cut with a freezing microtome into sections averaging 20 to 25 microns in thickness. As a rule, no difficulty was encountered in cutting the sections, for any calcified tissue that could be cut in the fresh state with a knife could be cut in the frozen state with a microtome. In handling the sections, however, densely calcified areas of the matrix often broke away. These remnants were discarded.

From the Department of Pathology of Cornell University Medical School.

Determination of Calcium in Sections of Cartilage.—The fresh frozen sections, weighing 300 to 600 mg., were washed in distilled water and then transferred to a volume of two-tenths molar acetate buffer solution, p_H 4.8, approximating 1 cc. for each 2 mg. of tissue. After decalcification at 5 C. for twenty-four hours, the sections were filtered from the buffer solution over a sintered glass disk, washed with distilled water, dried in vacuo and then dried to constant weight at 110 C. In this way the value for the quantity of decalcified cartilage was obtained.

In determining the quantity of calcium extracted from the cartilage, the calcium was precipitated from the buffer solution as calcium oxalate and the estimation completed by alkalimetric titration of the oxide.[1]

The values obtained for calcium by this method were in good agreement with those from control analyses of the ashes of selected samples.

Determination of Lipids.—Each dry decalcified lot of sections was extracted for twenty-four hours under a reflux condenser with a boiling solution containing equal parts of ethyl ether and petroleum benzine. The extract was reduced to dryness and the weight of the ether-soluble residue obtained.

Hydrolysis of Cartilage After Extraction of Calcium and Lipids.—After extraction of calcium and lipids, 125 mg. of cartilage was resected from each dry lot of sections. There were 24 samples, 21 of which were from different cases. Six samples were obtained from different pairs of costal cartilages in 3 cases. Each sample was placed in a long pyrex tube containing 9.5 cc. of 4.2 normal hydrochloric acid and 0.5 cc. of 10 per cent barium chloride. The tubes were sealed in a flame and the contents hydrolyzed for seven hours at 100 C.

Determination of Sulfate Liberated by Hydrolysis.—During hydrolysis, sulfate ions precipitated as barium sulfate. After completion of the period of hydrolysis, the sealed tubes were broken and an additional 0.25 cc. of 10 per cent barium chloride added to insure near quantitative precipitation. The hydrolysates were cooled and filtered. The residues were washed and ashed. The ash of each sample was weighed and the sulfate computed.

Determination of Reducing Substances in Hydrolysates.—One cubic centimeter of each hydrolysate was used for quantitative determination of reducing substances by the micromethod of Folin, modified as required by conditions.[2]

Determination of Iron in Hydrolysates.—Each hydrolysate, minus 1 cc. removed for analysis of reducing substances, was dried. The residues were ashed with nitric acid and a 30 per cent solution of hydrogen peroxide. Iron content was determined by the method of Lintzel.[3]

Estimation of Chondroitin-Sulfuric Acid.—The quantity of chondroitin-sulfuric acid in small amounts of cartilage can be estimated without great error if the quantities of reducing substances and sulfate in hydrolysates of the cartilage are known.[2] Approximately one-half the total quantity of reducing substances is derived from chondroitin-sulfuric acid. Essentially all sulfate recovered from hydrolysates of cartilage, previously extracted at p_H 5 to remove the labile non-diffusible fraction, is a degradation product of chondroitin-sulfuric acid. As it was advisable in the present study to work with a small amount of tissue, the

1. Fiske, C. H., and Adams, E. T.: J. Am. Chem. Soc. **53**:2498, 1931.
2. Hass, G. M., and Garthwaite, B.: Arch. Path. **33**:145, 1942.
3. Lintzel, W.: Ztschr. f. d. ges. exper. Med. **86**:269, 1933.

quantities of polysaccharide were routinely computed from data obtained by analyses of reducing substances and sulfate.

Because of the possible error in the indirect method of determining the quantity of polysaccharide, the conclusions from the microchemical data were checked by macrochemical semiquantitative isolation of the polysaccharide from tissues of selected cases. For this purpose, surplus cartilage from the patients in cases 8, 13, 18 and 22, aged 35, 47, 60 and 66 years respectively, was ground into fragments, dried by alcohol-ether extraction and then pulverized. The polysaccharide was isolated as the barium salt from 5 Gm. of each sample by the method of Levene.[4] One gram of each sample was ashed with nitric acid and a 30 per cent solution of hydrogen peroxide. Calcium was recovered from the ash as calcium oxalate and the quantity calculated from the weight of the ignited precipitate.

Morphologic Study.—A representative part of each block of cartilage used for chemical analysis was available for gross and microscopic study. In the gross study, records were made of the resilience, fragility, fibrillation, pigmentation and calcium deposits. The blocks were then fixed in 4 per cent solution of formaldehyde U. S. P., decalcified and embedded in paraffin. Permanent sections were stained with hematoxylin and eosin for microscopic study. In this study cells were counted in several peripheral and central fields of the matrix. Morphologic changes in the structures of cells were noted. Rough estimations of the percentage of the matrix occupied by osteoid tissue, bone, bone marrow and fibrillary or other disintegrative changes were made.

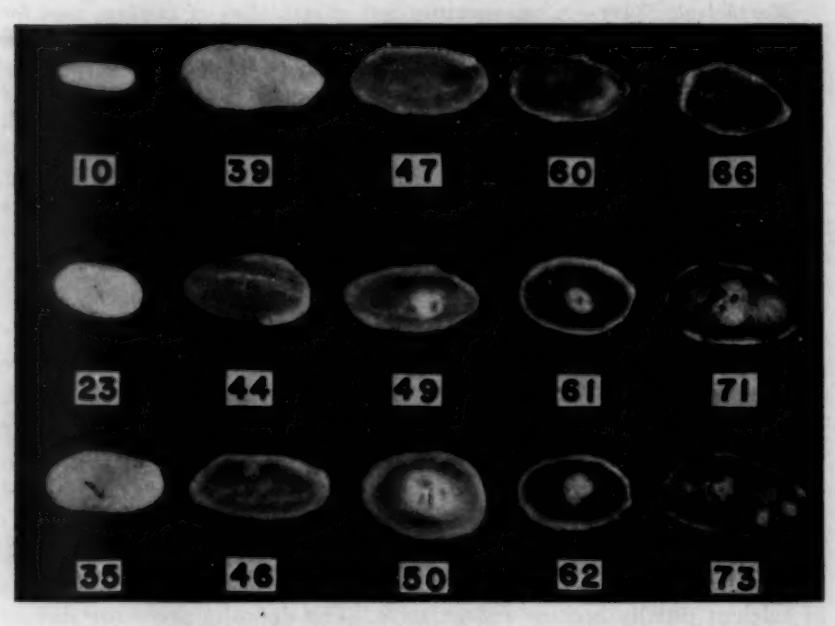
RESULTS

Gross Morphologic Changes.—From birth until middle life there is a progressive increase in the cross-sectional area of costal cartilage (figure, ages 10 to 39). During this period of growth the tissue is white, homogeneous, translucent and resilient. By the fourth decade of life several changes, especially in the axial region of the cartilage, begin to appear. There is a pale yellow pigmentation (figure, ages 35 to 44 years). The tissue is more opaque. Patches of linear fibrillary striations are found (figure, ages 44, 46 and 47). The resiliency is decreased. As these changes progress through the fourth and fifth decades, deposits of calcium usually become recognizable. The deposits are more diffuse than circumscribed and usually appear first in the pigmented central areas (figure, age 49). With increasing age the average amount of calcium increases, with partial restoration of white color to the tissue (figure, ages 49 and 50). Islands of bone and bone marrow appear, especially in densely calcified areas (figure, ages 61 and 62). Fibrillation and brown pigmentation, the former irregularly and the latter diffusely, spread from the axial area toward the perichondrium (figure, ages 35 to 62). The only cartilage which retains youthful appearance is that which lies just beneath the perichondrium (figure, ages 49 to 62). Even this at times is modified by atypical proliferation of the perichondrium, calcification and osteogenesis. Finally, in the eighth decade the central

4. Levene, P. A. T.: Hexosamines, Their Derivatives and Mucins and Mucoids, Monograph 18, Rockefeller Institute for Medical Research, 1922.

areas of cartilage often are occupied by irregular white deposits of calcium and circumscribed islands of bone (figure, ages 71 and 73). Elsewhere, the matrix, even beneath the perichondrium, is brown, fragile and often fibrillary (figure, ages 71 and 73).

Microscopic Morphologic Changes.—In young costal cartilage, there is continuous proliferation of cells of the perichondrium, with differentiation of the proliferating cells into chondrocytes and production of intercellular matrix. Hence, peripherally the ratio of cells to matrix is high, and this ratio decreases as the cells become enveloped in an abundant matrix (table 2). As maturity is reached, the proliferation of cells



Gross appearance of costal cartilage at various ages.

ceases and the ratio of cells to matrix approaches a minimum late in the third and early in the fourth decade of life. In this period several microscopic changes begin to appear. Granular deposits of calcium are present in the matrix, especially in metachromatic regions. Local parallel bundles of prominent fibrils are encountered. These are first recognized in the axis of cartilage in areas which are elective sites of initial pigmentation and calcium deposition. With increasing age, fibrillation usually becomes coarse and more widespread (table 2). There is a coincidental decrease in the number of cells, and those which remain often exhibit morphologic changes of diminishing viability. This is especially prominent in many areas where the homogeneous or fibrillated matrix has undergone disintegration. In these areas the normal orderly

TABLE 1.—*Chemical Analysis of Cartilage* *

Case	Sample	Age	Lipid, per Cent	Reducing Substances, per Cent	Sulfur as SO_4, per Cent	Iron, per Cent	Calcium, per Cent
10507	1	2	0.98	25.6	0.81	0.017	0.046
10450	2	10	0.07	28.6	1.00	0.021	0.062
10496	3	11	0.52	27.7	0.95	0.012	0.099
10563	4	12	0.04	27.1	0.86	0.028	0.155
10560	5	21	0.03	31.6	1.21	0.024	0.177
10524	6	23	0.11	31.6	1.46	0.004	0.278
10455	7	30	0.14	30.8	1.37	0.009	0.327
10567	8	35	0.08	30.3	1.30	0.010	0.255
10500	9	39	0.51	30.0	1.08	0.014	3.147
10462	10	39	0.12	29.7	1.37	0.015	0.257
10561	11	44	0.01	26.7	1.09	0.008	0.249
10498	12	46	0.12	26.9	1.18	0.008	0.481
10535	13	47	0.13	28.1	1.06	0.016	0.280
10388A	14	49	0.38	27.2	1.05	0.006	2.622
10388B	15	49	0.49	27.0	1.01	0.010	0.960
10389A	16	50	0.40	30.8	1.17	0.014	1.166
10389B	17	50	0.17	30.4	1.19	0.012	0.501
10385A	18	60	0.42	28.3	0.92	0.011	1.980
10385B	19	60	0.43	27.3	0.88	0.032	6.693
10400	20	61	0.45	23.6	0.85	0.021	2.414
10508	21	62	1.52	22.4	0.66	0.027	4.067
10504	22	66	0.13	24.6	0.68	0.011	0.758
10525	23	71	0.06	19.8	0.61	0.006	1.926
10529	24	73	0.11	24.3	0.80	0.024	1.186

* All values are expressed as percentages of ether-extracted decalcified tissues.

TABLE 2.—*Comparison of the Microscopic Appearance of Cartilage with the Amounts of Chondroitin-Sulfuric Acid and Calcium*

		Chondrocytes, Average Number per Unit Area		Percentage of Cross-Sectional Area				Percentage of Chondroitin-Sulfuric Acid* Estimated by;			
				Osseous		Disintegrations					
Sample	Age, Yr	Peripheral	Central	Matrix	Marrow	Fibrillary	Other Types	Reducing Substances	Sulfate	Chemical Isolation	Percentage of Calcium *
1	2	45	20	0	0	0	0	14.1	12.1	....	0.046
2	10	32	20	0	0	0	0	18.1	15.0	....	0.062
3	11	46	14	0	0	2	1	16.9	14.2	....	0.099
4	12	41	17	0	0	2	0	16.1	12.9	....	0.155
5	21	26	12	0	0	5	0	22.1	18.2	....	0.177
6	23	28	12	0	0	10	2	22.1	21.9	....	0.278
7	30	24	14	0	0	5	2	21.1	20.6	....	0.327
8	35	23	9	0	0	10	5	20.4	19.5	11.4	0.255
9	39	27	14	5	5	5	1	20.0	15.5	....	3.147
10	39	20	15	0	0	5	5	19.6	20.6	....	0.257
11	44	22	9	0	0	5	2	15.6	16.4	....	0.249
12	46	20	8	0	0	20	1	15.9	17.7	....	0.481
13	47	27	14	0	0	10	0	17.5	15.9	8.5	0.280
14	49	32	11	5	2	2	5	16.3	15.8	....	2.622
15	49	21	14	0	0	5	5	16.0	15.2	....	0.960
16	50	16	10	0	0	5	0	21.1	17.6	....	1.166
17	50	21	13	0	0	10	2	20.5	17.9	....	0.501
18	60	22	10	5	2	5	2	17.7	13.8	6.9	1.980
19	60	24	12	10	10	10	5	16.4	13.2	....	6.693
20	61	14	7	2	1	10	2	11.5	12.8	....	2.414
21	62	25	9	2	2	15	5	9.9	9.9	....	4.067
22	66	27	11	2	2	20	10	12.8	10.2	4.8	0.758
23	71	13	10	0	0	25	10	6.4	9.2	....	1.926
24	73	18	8	2	2	15	15	12.4	12.0	....	1.186

* The values are calculated as percentages of ether-extracted decalcified tissues.

structure is replaced by granular, globular and nondescript discontinuous acellular clumps of abnormal matrix (table 2). But in many fibrillary, densely calcified fields the cells often are well preserved. It is in these areas that osteoid tissue, bone and fatty bone marrow, more or less occupied by hemopoietic foci, appear. These new tissues develop, especially in axial densely calcified areas, but the organization is unlike that encountered in normal enchondral ossification centers, and they appear long after the time of closure of epiphysial lines (table 2).

Quantity of Lipids.—The quantities of ether-soluble substances are recorded in table 1. As indicated by the microscopic studies, these were extracted not only from cartilage but also from adventitious intracartilaginous islands of bone and bone marrow. The values range from 0.01 to 1.52 per cent. In comparison with the values from most human tissues these values are very low. Several high values are to be attributed to islands of fat cells in marrow spaces. Variations in values are also partly due to unavoidable contamination of cartilage with extracartilaginous adipose tissue during removal of the perichondrium. Hence, the low values in each age group are the more correct ones. The uniformity of these values indicates that the lipid content is independent of any of the observed changes in aging cartilage. Furthermore, it is improbable, in view of the constant low figures, that extracellular accumulations of lipids, which are often associated with calcification of other intercellular matrices, have any bearing on calcium deposition in cartilage.

Quantity of Iron.—The quantities of iron recovered from cartilage after removal of lipids and calcium are recorded in table 1. The values are low and are independent of age or changes in the matrix incidental to calcium deposition. It is probable that higher values would have been obtained by a study of the ash prior to decalcification. The present values show that if iron is implicated as a precursor of calcium in cartilage it is not firmly bound to cells or matrix.

Quantity of Pigment.—The supposition that pigmentation of the matrix is due to the presence of lipids or iron was not confirmed by analysis. The pigment was not extracted with ether or with a buffer solution, p_H 4.8. Neither was there any relationship between the intensity or the quantity of pigmentation and the quantity of iron. Nor was the pigment detected microscopically as one of the "wear and tear" pigments often found in the cytoplasm of cells of old people.

The quantity of pigment in cartilage increases with age. It first appears in axial parts of cartilage that are destined to undergo calcification and in time gradually spreads throughout the tissue. Speculation indicates that it may be a degradation product of chondroitin–sulfuric acid, for it appears as the polysaccharide decreases in quantity. The

possible role of the pigment in the promotion of calcification deserves further consideration.

Quantity of Calcium and Bone.—The data in table 2 show that the quantity of calcium increases with age. This increase begins in the first decade and progresses, more rapidly in some than in others, to reach high values in all cartilage over 50 years of age. When high values were encountered in young cartilage, there was ossification (table 2). This was also the rule when the quantity in aged tissue was unusually great. In general when the content of calcium at any age was greater than 1 per cent, there was ossification. This rule could not be strictly applied, because of variations in the volume of matrix occupied by a given amount of calcium, but it confirmed the impression that ossification did not begin until a large quantity of calcium had been concentrated in the area. In other words, in the 4 samples of cartilage with the highest calcium values, the amount of bone, even if fully calcified, would account for only a minor fraction of the calcium.

Quantity of Chondroitin-Sulfuric Acid.—The microchemical and random macrochemical estimations of the quantities of chondroitin-sulfuric acid were in good agreement though, as expected, the yields of purified polysaccharide were much lower than the predicted yields (table 2). This may be attributed to inevitable losses incidental to isolation and purification of the product.

The independent estimations of the content of polysaccharide by use of data referable to reducing substances and sulfate were in fair agreement (table 2). In accordance with conclusions from previous studies, about one half of the maximum quantity of reducing substances was derived from sources other than chondroitin-sulfuric acid.[2] Hence, about 15 per cent must be subtracted from each experimental value in order to obtain the percentage derived from the polysaccharide. The theoretic yield of reducing substances from chondroitin-sulfuric acid is about 75 per cent by weight in terms of dextrose. Data obtained by use of this figure and corrected values for reducing substances show that the amounts of chondroitin-sulfuric acid range from 6.6 to 22.1 per cent. A corresponding calculation with values for sulfur recovered as sulfate indicates that the amounts of the polysaccharide vary from 9.2 to 21.9 per cent. The amounts recovered by semiquantitative isolation varied from 4.8 to 11.4 per cent. All estimates are recorded in table 2.

In general, the quantity is low in infancy. It progressively increases to a maximum in the third and fourth decades of life. Thereafter, it decreases so that in the late seventh and eighth decades the content is less than half maximum values. With this decrease there are numerous simultaneous changes in the cartilage. Among these changes, a progressive increase in the amount of calcium is encountered, though no

quantitative relation seems to exist between the depletion of polysaccharide and the deposition of calcium. Nor is there any established quantitative relation between the depletion of polysaccharide and any gross or microscopic change. If any does exist, a revision and refinement of method will be required before it can be clearly exposed. This analysis is not beyond the scope of available histochemical micromethods.[2]

COMMENT

Physicochemical studies have shown that the deposition and the resorption of the calcium salts are regulated within limits by changes in special ionic concentrations in circulating bodily fluids. But this is a restricted regulation and fails to account for the fact that calcium salts are deposited neither diffusely nor indiscriminately but in elective sites. Among these elective sites, the matrix of cartilage is perhaps the best example, and it is the object of the present study. Costal rather than epiphysial cartilage was chosen for study because in it calcium is deposited in a simple tissue with minimal complication by formation of osteoid tissue and bone. Morphologic studies were planned so that an idea was obtained of the structure of each sample of cartilage subjected to chemical analysis. Analyses for iron and lipid were made to test the validity of theories which implicate these materials in the mechanism of calcification and to inquire into the possible relation of these materials to the brown pigmentation of aged cartilage. The estimation of the content of chondroitin–sulfuric acid was made because the large amount of this compound in normal uncalcified cartilage is the chemical characteristic by which cartilaginous matrices are best distinguished from avascular intercellular matrices, which do not ordinarily calcify.

The lipid content throughout life is constantly at such a low level that the conclusion is reached that lipids bear no quantitative relation to any observed change in the morphologic appearance or the chemical composition of cartilage.

The iron content of cartilage after extraction at p_H 4.8 is very low and relatively constant throughout life. The data justify no conclusions with respect to the participation of iron in calcification or in pigmentation of the matrix.

The amount of chondroitin–sulfuric acid is variable. The low value in early life is to be attributed to the low ratio of matrix to cells. As this ratio increases, the amount of polysaccharide increases up to a maximum in the third or the fourth decade of life. At this age, despite further increase in the ratio, the quantity of polysaccharide begins to decrease, calcium deposition is accelerated in the axes of the cartilages and morphologic changes begin to develop. Generation of chondrocytes by the perichondrium is inconspicuous. Signs of diminished viability are detectable in cells located deep in the matrix. Pale yellow pigmen-

tation and occasional foci of fibrillation appear in central axial areas. With increasing age all changes are accentuated. The quantity of polysaccharide falls to less than half maximum values. Calcium deposits increase in density, especially centrally, spreading outward in diminishing concentration toward the perichondrium. Small foci or osteogenesis appear, particularly in the dense areas of calcification. Generation of chondrocytes ceases. Cells everywhere, though they show remarkable persistence, display the usual morphologic changes of diminished viability. The light yellow pigmentation changes to brown and spreads throughout the tissue except in densely calcified areas. Areas of fibrillation are often prominent. The tissue is firm and brittle. This is the usual status in the eighth decade of life.

These data constitute an incomplete histochemical description of calcification rather than an explanation of the process. The description reveals morphologic and chemical coincidences in sequence but fails to specify causes and effects. However, the following speculations are in accord with the observations. So long as the polysaccharide content of cartilage remains at a high level, the amount of calcium in the matrix remains at a low level, although calcium is acquired in limited quantities during the period in which there is an apparent increment of polysaccharide. As the quantity of polysaccharide decreases with increasing age, there is a comparable, though variable, increase in calcium in the matrix. But this is not the only accompaniment of disappearance of polysaccharide. The number of cells in the matrix diminishes, and those which remain display morphologic changes which are ordinarily accepted as evidence of diminished activity, if not of decreased viability. But the functional capacity of those cells which persist in considerable numbers may still be very important from the standpoint of calcification. There is little doubt that one function of the cells is synthesis of polysaccharide or its precursors, and it is reasonable to assume that the limitation of this function in aged cartilage is responsible for the low polysaccharide values. In other words, the preservation of matrix in a youthful state is contingent on the continuous replacement of polysaccharide, and if this does not occur, the quantity of polysaccharide decreases. With the decrease, the increased rigidity, friability, fibrillation and pigmentation of the tissue become evident. Indeed, it is probable that fibrillation of the matrix is simply an exposure of fused elementary fibrils by disappearance of interfibrillar polysaccharide. A similar argument applies to the cause of loss of resilience of the matrix. The composition, source and significance of the pigment are unknown. It increases as polysaccharide decreases and appears first in the axial zones which are elective sites of calcification. It may be a degradation product of polysaccharide and unrelated to the mechanism of calcification, but

because of the spatial and temporal coincidence of pigmentation and calcification the pigment deserves further study.

Finally, the conclusion is reached that the mechanism by which the intercellular matrix is maintained in a healthy state is the mechanism which fails and in failing leads to a succession of complex changes. The concrete chemical evidence of this failure is not calcification primarily but is depletion of chondroitin–sulfuric acid. Indeed, one oversimplified interpretation of the data is that maintenance of a high level of chondroitin–sulfuric acid is a device by which cartilage is protected against calcification.

SUMMARY

From infancy until the fourth decade of life the amount of chondroitin–sulfuric acid in costal cartilage increases in proportion to the increase in the ratio of matrix to cell. In later life, despite further increase in the ratio, the amount of polysaccharide decreases. Coincidental with this decrease, several changes appear, especially in the central areas of the cartilage. Calcium is deposited. A diffuse yellow pigment is detectable. Foci of fibrillation develop. The rate of differentiation of chondrocytes from the perichondrium decreases, and cells located deep in the matrix show early signs of diminished viability. With increasing age these changes become more conspicuous, so that in the eighth decade the amount of chondroitin–sulfuric acid has been reduced to less than half the maximum value. Calcium deposits are prominent. Islands of tissue disintegration, osteoid matrix and bone are often present, especially in densely calcified fields of the cartilaginous matrix. The yellow pigment, which is neither lipid nor iron, now contributes a brown tinge to all cartilage except the white densely calcified areas. The foci of fibrillation, initially central in location, are more numerous and widely distributed. There is no proliferation of chondrocytes from the perichondrium, but most mature cells throughout the tissue persist despite long-continued signs of diminished viability.

The spatial and temporal relationships among these progressive changes indicate that calcification of cartilage in the presence of an appropriate supply of necessary ions depends on failure of the mechanisms responsible for maintenance of the intercellular matrix.

Case Reports

MYCOTIC AND DISSECTING ANEURYSMS OF THE AORTA COMPLICATING BACTERIAL ENDOCARDITIS

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A dissecting aneurysm resulting from a mycotic aneurysm in the case reported here represents a rare complication of bacterial endocarditis. Because of the unusual combination of lesions it seems pertinent to put this case on record.

REPORT OF A CASE

W. R., a 75 year old man, was admitted to the Boston City Hospital on Jan. 22, 1940, because of inability to void urine since a few days before. He was irrational and unable to give a lucid history.

When examined, he was thrashing about in bed. The lungs were normal. The heart was enlarged to percussion, and a loud systolic murmur was heard over the precordium. No diastolic murmur was heard. The prostate was generally enlarged and firm. The penis was absent, the urethral opening being level with the abdominal wall. There was slight pitting edema of the legs. The blood pressure was 122 systolic and 58 diastolic.

The hemoglobin content was 88 per cent, and the total leukocyte count was 21,800 per cubic millimeter. The urinary sediment showed 20 to 25 white blood cells per high power field.

The patient remained irrational and incontinent. Rales developed at the bases of both lungs with a concomitant rise in temperature. Death occurred on the sixth hospital day.

The final clinical diagnoses were: arteriosclerotic heart disease with mild decompensation; pyelonephritis; operative absence of the penis; benign prostatic hypertrophy, and intertrigo.

Postmortem Examination.—Externally the body was that of a well developed and well nourished elderly white man. There was an umbilical hernia, 2.5 cm. in diameter and 0.8 cm. in height. The skin over the legs was purple and scaly. The penis was absent, having been amputed at the level of the penoscrotal junction. The peritoneal cavity was without pathologic change, and each pleural cavity contained about 400 cc. of clear straw-colored free fluid.

The pericardium contained about 300 cc. of partly clotted blood. There was no demonstrable perforation in the heart or in the large vessels associated with

From the Mallory Institute of Pathology of the Boston City Hospital.

the pericardial cavity. The blood was presumably derived by slow leakage from the adventitia of the ascending aorta, which showed the bluish purple mottling characteristic of hemorrhage. The heart was somewhat enlarged, weighing 520 Gm.

The aortic valve was the site of greatest interest (fig. 1), exhibiting the characteristics of acute bacterial endocarditis involving adjacent portions of the left posterior and anterior cusps. The left posterior cusp exhibited a friable papillary gray and brown vegetation, measuring 1.5 cm. by 0.5 cm., well attached

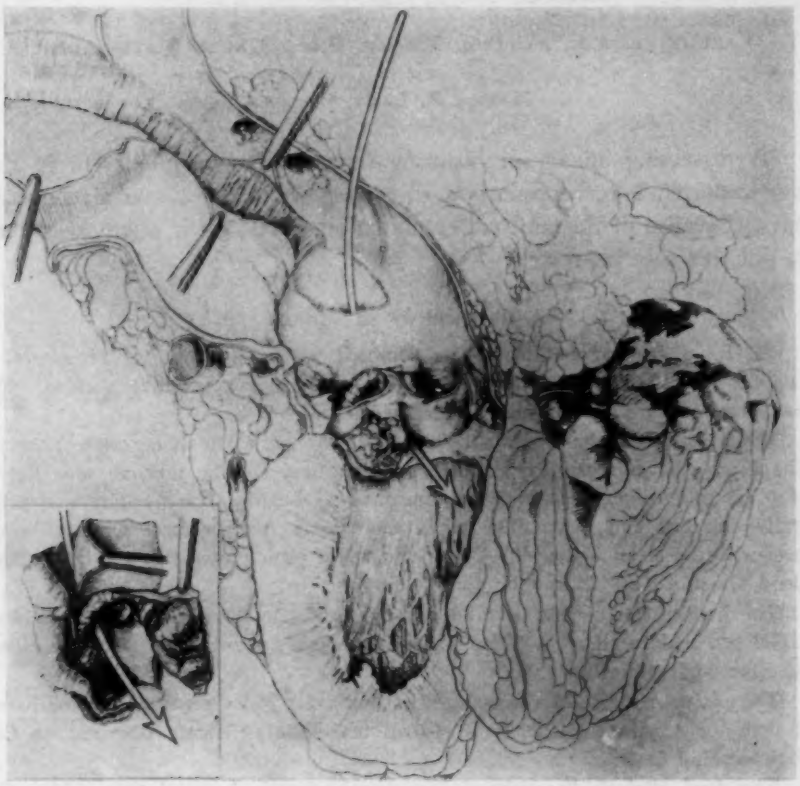

Fig. 1.—Drawing of heart and aorta. The heart and aorta have been opened, exposing the left ventricular chamber and the aortic valve. Two cusps of the aortic valve show the vegetations of acute bacterial endocarditis with ulceration of the commissure between these two cusps. A dissecting aneurysm is present in the aorta. In the insert the anterior aortic leaflet has been reflected, exposing an ulcerated lateral wall of the corresponding sinus of Valsalva. At the upper limit a mycotic aneurysm is present. This is continuous with the dissecting aneurysm as shown by the arrow.

to the sinus surface of the anterior one quarter of the cusp. Beneath this the leaflet was ulcerated through to the contact surface. The anterior leaflet showed a process similar in kind but more extensive, so that papillary vegetations projected from the sinus as well as from the contact surfaces, and fully half of

the cusp was involved. There were several points of ulceration through the cusp associated with complete detachment of the cusp from its left commissural attachment. The ulcerative process extended to the commissure which lay between the two cusps described. In addition the wall of the anterior sinus of Valsalva showed considerable ulceration; no recognizable aortic tissue was present, and there was a moderate degree of aneurysmal dilatation of this sinus. This ulcerative process extended upward on the aortic wall opposite the anterior leaflet to a level just above that of the free margins of the cusps. The uppermost limit of the aortic lesion was demarcated by a row of papillary friable vegetations on the aortic wall. Just beneath these in the aorta there was an ovoid defect, measuring 1.2 cm. by 0.5 cm., through which the lumen of the anterior sinus of Valsalva communicated with a typical intramedial dissecting aortic aneurysm. The defect lay between the level of the left attachment of the anterior leaflet and the origin of the right coronary artery. The dissecting

Fig. 2.—Left subclavian artery. The aortic dissecting aneurysm has extended into the left subclavian artery. The intramedial hematoma is responsible for considerable narrowing and for the crescent shape of the original lumen of the vessel.

aneurysm arose from the base of the aforementioned defect to involve the entire length of the aorta and extended into each common iliac artery. The dissection continued from here to involve from 1 to 3 cm. of the external and internal iliac arteries without rerupturing into the lumens of these vessels. The dissection also extended into the great vessels arising from the aortic arch. It rose to the level of the bifurcation in the innominate artery, for a distance of 3 cm. in the left common carotid artery and 4 cm. in the left subclavian artery (fig. 2).

The intima of the entire aorta above the root showed only a mild degree of atherosclerosis and no point of rupture.

In addition to the formation of the mycotic aneurysm at the level of the endocarditis, there was extension of the inflammatory process through the sub-

stance of the heart, the lesion appearing on the medial wall of the right auricle as a bulging reddish brown area, 1.8 cm. in diameter.

The anterior leaflet of the mitral valve showed at its base a mild degree of yellow thickening characteristic of arteriosclerosis. Otherwise the valves showed no definite evidence of any disease existing prior to the bacterial endocarditis.

The kidneys were of usual size, the combined weight being 370 Gm. The capsules stripped with ease to reveal numerous flat-based depressions typical of healed pyelonephritis and averaging 1.5 cm. in diameter. The cortical tissue between these depressions averaged 0.8 cm. in thickness and was not remarkable. There were no apparent renal hemorrhages or infarcts.

The spleen was slightly enlarged, weighing 280 Gm., and exhibited the soft, semisolid consistency frequently seen in sepsis. There were no infarcts.

The right adrenal showed two hematomas, one measuring 3.7 cm., the other 0.9 cm. in diameter. The lungs showed a moderate degree of congestion and edema. The liver was moderately enlarged and showed the yellow color characteristic of fatty metamorphosis. There was benign prostatic hypertrophy. Examination of the gastrointestinal tract revealed several small diverticula, measuring 0.7 cm. in diameter, in the sigmoid. The brain, the pancreas and the vertebral marrow were normal on gross examination.

Microscopic Examination.—The anterior sinus of Valsalva showed beneath the surface of its lateral wall considerable ulceration with necrosis, cellular infiltration and abscess formation (fig. 3). On the surface were deposited large collections of fibrin heavily infiltrated with polymorphonuclear leukocytes. At the upper level of this sinus the aorta was involved by an acute ulcerative process extending through the intima deeply into the wall, giving rise to a separation of the media into two layers of unequal thickness. The thicker was attached to the intima while the thinner, representing about one sixth of the medial thickness, was attached to the adventitia.

This separation represented the origin of the dissecting aneurysm described in gross. In only the lower part of the separation was there an inflammatory process. Above this the separated surfaces showed none and undoubtedly represented a tearing of the media by a stream of blood coming from the lower inflammatory area, which may be considered a mycotic aneurysm.

Bacteriologic Examination.—Culture of the blood from the right auricle of the heart and of the thrombus in the left common carotid artery produced no growth. Bacillus coli communis was obtained from the liver.

Cultures of the vegetation on the aortic valve and of the spleen and the kidney revealed pure growths of a nonmotile organism which on smear was a gram-negative pleomorphic bacillus with clubbed ends. It fermented dextrose, lactose, saccharose, xylose and mannite with the formation of acid but no gas. Even when it was transferred daily for three weeks in dextrose it did not form gas. Neither gas nor acid were formed when the organism was grown in inositol.

The reactions of the organism to certain special tests were as follows: methyl red reaction, negative; Voges-Proskauer reaction, negative; indole reaction, positive; ammonia formation, negative; catalase, positive.

Diagnosis.—The conditions finally diagnosed were: acute bacterial endocarditis with mycotic and dissecting aneurysms of the aorta; hemopericardium; hydrothorax; pulmonary congestion and edema; fatty infiltration of the liver; diverticulosis of the sigmoid; acute and healed pyelonephritis; benign prostatic hypertrophy; hematomas of the right adrenal, and umbilical hernia.

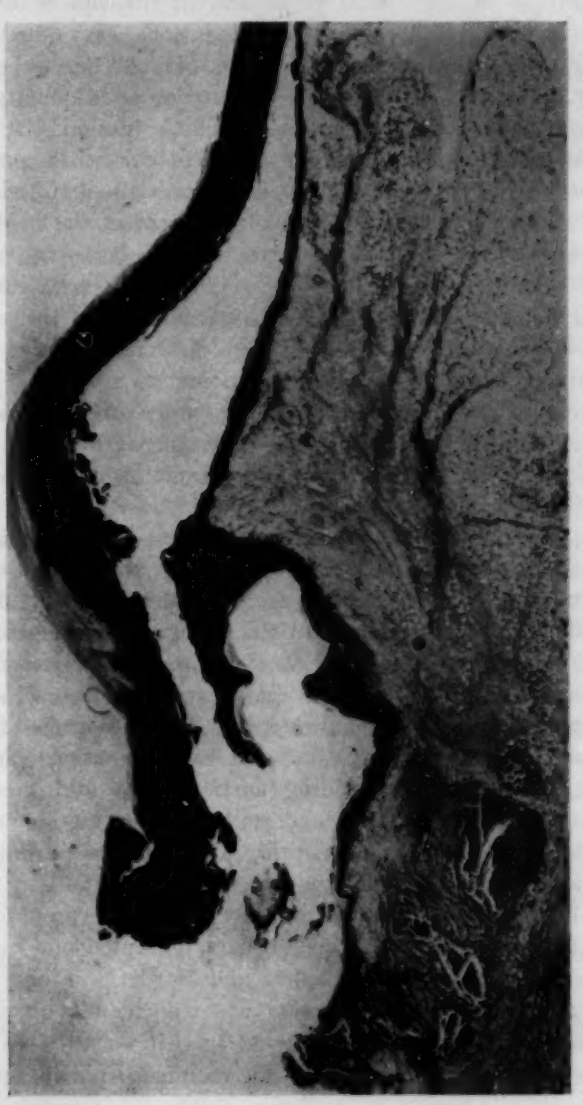

Fig. 3.—Lateral wall of the anterior sinus of Valsalva. In the lower portion of the photomicrograph the lateral wall of the sinus is shown ulcerated. The inflammatory process has extended through the intima into the media and is continuous with the aortic dissecting aneurysm which lies in the upper half of the illustration.

COMMENT

Dissecting aneurysm of the aorta arising as a complication of bacterial endocarditis is a rare condition. Cases of dissecting aneurysm of the aorta may be divided into two groups: (1) those in which a primary disease of the intima is present at the site of origin of the dissection and (2) those in which the intima shows no evidence of abnormality present prior to the rupture. The second group is by far the larger [1] and in these cases the aneurysm is frequently associated with clinical and pathologic evidence of hypertension. In this group, postmortem examination shows no evidence of intrinsic disease of the intima but may show focal defects in the media, often referred to as idiopathic medionecrosis. Syphilitic involvement of the media is rarely seen in cases of dissecting aneurysm, and the opinion has been expressed [2] that the medial scars of syphilis may act as a protection against the development of a progressive hematoma of the aortic media.

Shennon [1] has indicated that although dissection starting at the base of an atheromatous ulcer has been mentioned as a common occurrence, this lesion occurred only seldom in the series of cases he reviewed. Thus, in a group of 218 cases of dissecting aneurysm of the aorta there were only 6 in which there was a definite statement that the dissection began in the base of an atheromatous ulcer.

Even more unusual is dissecting aneurysm of the aorta arising in the base of a mycotic aneurysm. We have been able to find only 2 cases in the recent literature. The first is the case reported by Lanbry and Bordet.[3] The patient, a man 40 years of age, gave a past history of rheumatic fever. During his terminal illness he was febrile, and his clinical course suggested bacterial endocarditis. Two blood cultures were negative, and one showed a tetragen. The patient died from a large hemopericardium. At autopsy the heart had vegetations on the mitral and aortic valves. The ascending aortic intima and media showed large fissures which communicated with a dissecting aneurysm lying between the media and the adventitia. The authors did not report the microscopic features and did not mention bacteriologic findings at autopsy. It is therefore difficult to accept the interpretation that the endocarditis was bacterial. From the data presented one might justifiably assume that the case of Lanbry and Bordet was one in which a dissecting aneurysm of the usual type was coincident with endocarditis.

The second case was that reported by Lippincott.[4] In a 59 year old man with synovitis of the knee due to infection with Streptococcus

1. Shennon, T.: Dissecting Aneurysms, Medical Research Council, Special Report Series, no. 193, London, His Majesty's Stationery Office, 1934.

2. Weiss, S.: New England J. Med. **218**:512, 1938.

3. Lanbry, C. H., and Bordet, F. R.: Bull. et mém. Soc. méd. d. hôp. de Paris **47**:179, 1923.

4. Lippincott, S. W.: Canad. M. A. J. **43**:115, 1940.

haemolyticus of the beta type, septic aortitis of the lower thoracic part of the aorta developed from which a typical dissecting aneurysm arose. Bacterial endocarditis was not present in this case.

In cases of bacterial endocarditis one or more foci of acute inflammation may develop in the aortic intima with extension of the process into the deeper layers of the vessel wall and subsequent formation of a mycotic aneurysm. Usually death is due to the endocardial inflammatory process with embolic phenomena, and the mycotic aneurysm is an incidental finding at autopsy. Less commonly, the mycotic process may invade the aorta so deeply that rupture through the adventitia occurs and the patient dies of massive hemorrhage into one of the body spaces. Only rarely, as we have already indicated, does a typical progressive intramedial hematoma or a dissecting aneurysm originate at the base of a mycotic aneurysm.

The organism cultured from the valvular vegetation, the spleen and the kidney undoubtedly represents the causative organism of the endocarditis and mycotic aneurysm. We have not been able to classify this bacillus because of the extremely unusual combination of its reactions. While it is a lactose fermenter, its inability to form gas with the carbohydrates, together with its negative methyl red and Voges-Proskauer reactions, rules it out of the colon bacillus class. The lack of encapsulation, the failure to form gas, the negative methyl red and positive indole reactions and the failure to ferment inositol and to form ammonia serve to distinguish it from the Friedländer group. The organism could be distinguished from members of the dysentery group of bacilli by its prompt lactose-fermenting quality and its failure to reduce nitrates and to form ammonia. Absence of gas rules out the paratyphoid group.

For the time being the organism is given the broad classification of coliform lactose-fermenting bacillus.

SUMMARY

The case of a 75 year old man with acute bacterial endocarditis of the aortic valve, in whom a dissecting aneurysm of the aorta arose in the base of a complicating mycotic aneurysm is presented.

The etiologic organism was a pleomorphic gram-negative lactose-fermenting bacillus that could not be classified accurately.

The case is unusual from several angles, namely, (1) the association of bacterial endocarditis with dissecting aortic aneurysm, (2) the development of dissecting aneurysm in the base of a mycotic aneurysm and (3) the type of organism responsible for the endocarditis.

MESOTHELIOMA (ENDOTHELIOMA) OF THE PERITONEUM

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Highly characteristic conditions are produced in the peritoneum and in the pleura by primary tumors developing in these tissues.[1] The same is true for tumors of the pericardium.[2]

Considerable discussion has arisen in the literature as to whether these growths should be termed "endothelioma" or "mesoendothelioma," and recently "mesothelioma" is coming into use.

The majority of the authors are less impressed by embryologic considerations than by the morphologic and physiologic aspects of the tumor cells and by their derivation from serosal cells.

Some doubt has been expressed by several observers as to whether a true primary neoplasm ever arises from these membranes, most of the reported ones being considered as secondary carcinoma. It is not difficult to understand this contention, as these tumors do assume characteristics that cause them to resemble adenocarcinoma and some of the reported ones may have been of this nature.

We do not intend to enter into this controversial subject, and no attempt is made to review the somewhat meager and confused literature. We wish to report a case which, after thorough investigation at autopsy and careful study of many sections from involved areas, we believe to represent a true primary neoplasm arising from the serosal cells of the peritoneum.

We have adopted the name "mesothelioma," which seems best to express the origin and the nature of this neoplasm.

REPORT OF A CASE

A white woman 62 years of age entered the hospital Oct. 2, 1940. She had been sick for about eight weeks with nausea and attacks of vomiting; she also complained of weakness and pains in the right lower quadrant of the abdomen. The pain was accentuated on standing or walking. Bearing-down sensations were present in the region of the bladder, and some pain was present during micturition.

She was an extremely obese woman. She did not appear to be in acute distress. The heart was enlarged in all diameters; the liver was also somewhat enlarged and extended about 4 cm. below the right costal margin. Slight icterus developed, and projectile vomiting with abdominal distention. The temperature became septic. The red blood cell count was 4,540,000; the hemoglobin content, 82 per cent; the white cell count was 14,000, and the differential count was polymorphonuclears 78 per cent, juvenile forms 12 per cent and lymphocytes 10 per cent. The urine was normal.

From the Department of Pathology of St. Vincent's Hospital.

1. Ewing, J. E.: Neoplastic Diseases, ed. 4, Philadelphia, W. B. Saunders Company, 1940, pp. 356-359.

2. McDonald, S., Jr.: J. Path. & Bact. 43:137, 1936.

Roentgen examination revealed the following facts: The nonvisualized gallbladder contained many stones. Diverticula were present in the colon. There was a small supraphrenic pouch. No defects were present in the stomach. Peristalsis progressed to the pylorus normally. The duodenal cap could not be visualized. After twenty-four hours there was no residue in the stomach; the barium sulfate was distributed throughout the colon, a small amount remaining in the terminal part of the ileum. The chest showed increased density at the left base. The cardiac shadow was definitely enlarged in all diameters; the retrocardiac space was not narrowed, however, as there was a definite increase in the dorsal kyphosis making the retrocardiac space somewhat wider than normal. The aorta appeared normal; the esophagus was normal.

The septic temperature continued; myocardial failure developed, and the patient died Nov. 20, 1940.

Fig. 1.—A portion of the involved peritoneum over the colon and a portion of the ileum with mesentery. The resemblance to plastic fibrinous peritonitis is well seen, together with large nodular areas.

Autopsy.—There was slight icterus, and the abdomen was somewhat distended; other external findings were not important.

The mouth, tongue and oropharynx appeared normal. The larynx, trachea and bronchi down into the smaller branches into the lungs showed no changes. The lungs were hyperemic and edematous and practically filled the pleural cavities. There was about 100 cc. of slightly turbid yellowish fluid in each pleural cavity. The pleural surfaces were smooth and glistening. There were no adhesions. There were no gross areas of consolidation in the lungs. There was 250 cc. of greenish yellow fluid in the pericardium. The heart appeared slightly smaller than normal; the musculature, valves and vessels showed no gross changes. The large vessels and other mediastinal structures, including the glands, were normal. The thymus gland was lost in the mediastinal fat.

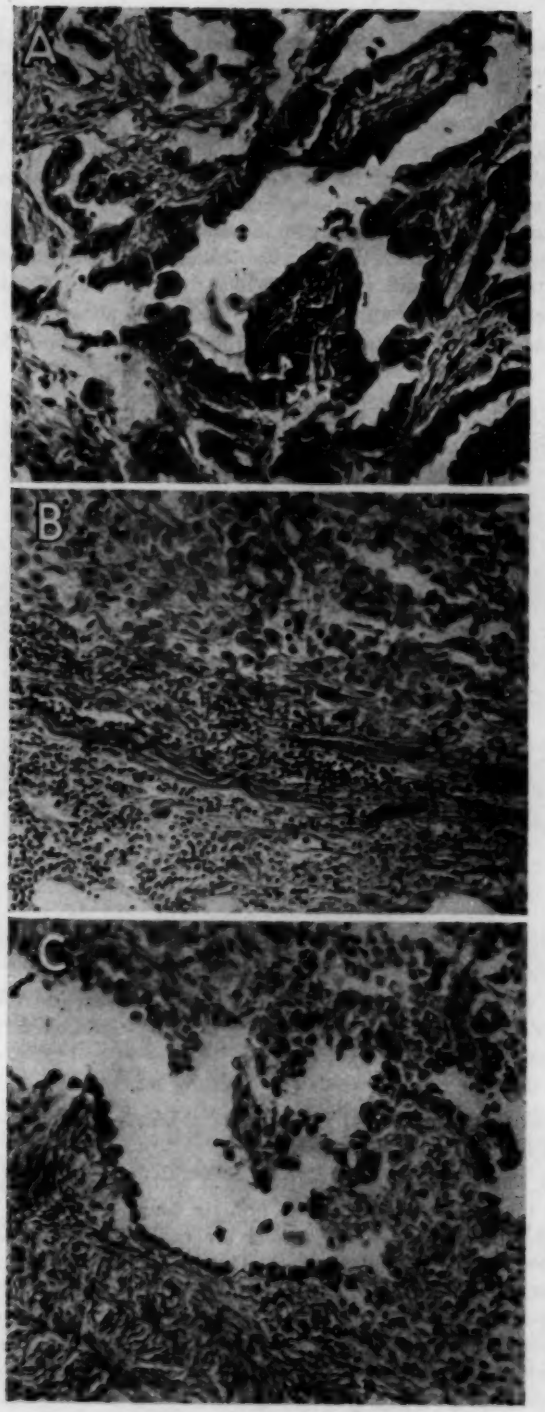

Figure 2

(See legend on opposite page)

There was 6 liters of slightly turbid yellowish fluid in the peritoneal cavity. The liver and spleen were high under the diaphragm. The liver was not enlarged; its substance was firm, light greenish yellow and mottled. The gallbladder was small and contracted around several faceted stones. The other biliary passages and vessels showed no gross changes.

The spleen was smaller than normal and presented chronic passive hyperemia. The pancreas was normal. The kidneys were about normal in size; the capsules were not adherent. A few small subcapsular cysts were present in each kidney. The renal pelvis, ureters and urinary bladder showed no gross changes. The adrenal glands showed autolytic changes. There was a large area of hemorrhage in the right perirenal fatty tissue extending retroperitoneally downward into the pelvic region. The renal vessels on this side showed no apparent gross lesions. The uterus, tubes and ovaries were normal except for the changes in the peritoneal coverings. One hemorrhagic cyst was present in the left ovary. The stomach, the upper part of the colon and some loops of the small intestine showed gaseous distention. The serosa over the lower parts of the intestines, including the mesentery, presented a thickened rough and nodular appearance. This was quite marked in the pelvis, extending over the fundus of the uterus and including the tubes and ovaries. Some areas presented the appearance of plastic peritonitis. Numerous adhesions were present between the intestinal surfaces so that they were matted in nodular masses. In some areas small vesicular nodules were seen over the margins of the neoplasm.

The stomach and intestines, including the rectum, were normal except for the changes just described. The appendix situated retrocecally was normal.

The dura was smooth and glistening. No gross changes were found in the brain or its membranes. The brain stem, as well as the cerebellum, was grossly normal. The blood vessels and the nerves at the base of the brain showed no gross changes. There was no neoplasm in the cranial cavity.

Microscopic Examination.—Sections from the lesions over the serosal surfaces of the uterus, ovaries, small intestines and mesentery showed essentially similar findings.

There was a more or less thickened layer of loose areolar vascularized connective tissue infiltrated with lymphocytes and plasma cells (fig. 2 *B*). Overlying this were irregular masses and groups of ovoid cells, ranging in type from cuboidal to low columnar, with slightly basophilic cytoplasm and large pale vesicular nuclei. There was a definite tendency toward the formation of small papilliferous projections from the surface growth, also toward the development of an alveolar arrangement, so that one might be impressed with the resemblance to carcinoma (fig. 2 *A* and *C*).

EXPLANATION OF FIGURE 2

A, area showing alveolar arrangement of the tumor cells with a supporting framework of connective tissue. From such areas a false diagnosis of adenocarcinoma might be made. × 100.

B, area from the serosal surface of a loop of the ileum at the margin of the mesenteric attachment. The loose areolar stroma is infiltrated with lymphocytes. × 100.

C, area showing ovarian cortical stroma with its overlying layer of serosal cells. The transformation from normal to neoplastic cells is seen at the lower center and to the right. × 100.

Careful observation, however, revealed the true nature of this tumor. In the larger nodular areas one could differentiate two types of stroma: a denser fibrous type without reticulum and a finer fibrillar form in which a definite reticulum was present (fig. 3). These reticulum fibers surrounded small groups of the tumor cells, acting as a supporting stroma, but did not surround or enter into the individual cells.

In sections in which a continuous surface lining was seen the cells showed rounded-out bulging free surfaces with deep indentations between them, so that the free border acquired a serrate appearance. This appearance is rather characteristic of tumors developing from mesothelium and is strikingly different from the appearance of developing carcinoma. In the sections taken from the growth over the ovary there was a definite transition from normal ovarian serosal cells to neoplastic cells, as demonstrated in figure 2 *C*.

Where the nodules were larger and more stroma was present, there was a tendency toward infiltration, and more changes were noted in the cell nuclei, more anaplasia was present, and some cells contained two or three nuclei. In the areas where little underlying stroma was present, there was no invasion but more of a tendency to grow away from the surface and to form papillary projections.

An occasional small area showed the cells with a fine fibrillar stroma forming small vesicle-like projections from the serosal surface. These findings were therefore so representative of a growth of the serosal cells that we feel that carcinoma can be excluded.

Mucin stains of these tissues gave negative results. No mucin was present in the peritoneal fluid.

COMMENT

Miller and Wynn [3] reported a case in which they noted two types of connective tissue—a well developed fibrous tissue in bands carrying vessels, mostly well formed, and constituting the supporting stroma, and a finer fibrillar type seemingly surrounding the tumor cells, forming a felted network. They suggested that the fibers appeared to be developed from the tumor cells. In their case there was a mucinous ascitic fluid which they stated was secreted by the tumor cells. We are able to confirm the findings of Miller and Wynn in that there are two types of connective tissue present in these tumors. A study of reticulum stains shows one type, minus reticulum, forming the denser and vascular stroma, the other, containing reticulum and forming a network around and between groups of the tumor cells. We cannot confirm their opinion that these fibers arise from and can be followed into the tumor cells.

The denser reticulum-negative stroma makes up the larger part of the nodules present in some areas of the growth.

We are also of the belief that the true tumor cells derived from the peritoneal serosa secrete no mucin. Mucin stains were negative in our case. Miller and Wynn thought that in their case the mucin present in the peritoneal fluid was secreted by the tumor cells. Hamdi and others [4] stated that in their cases ("adeno-coelothelioma") there was no tendency toward generalization and that no metastases were encountered.

3. Miller, R. T., and Wynn, W. H.: J. Path. & Bact. **12**:267, 1908.

4. Hamdi, H.; Louthai, M., and Schevket: Beitr. z. path. Anat. u. z. allg. Path. **19**:441, 1928.

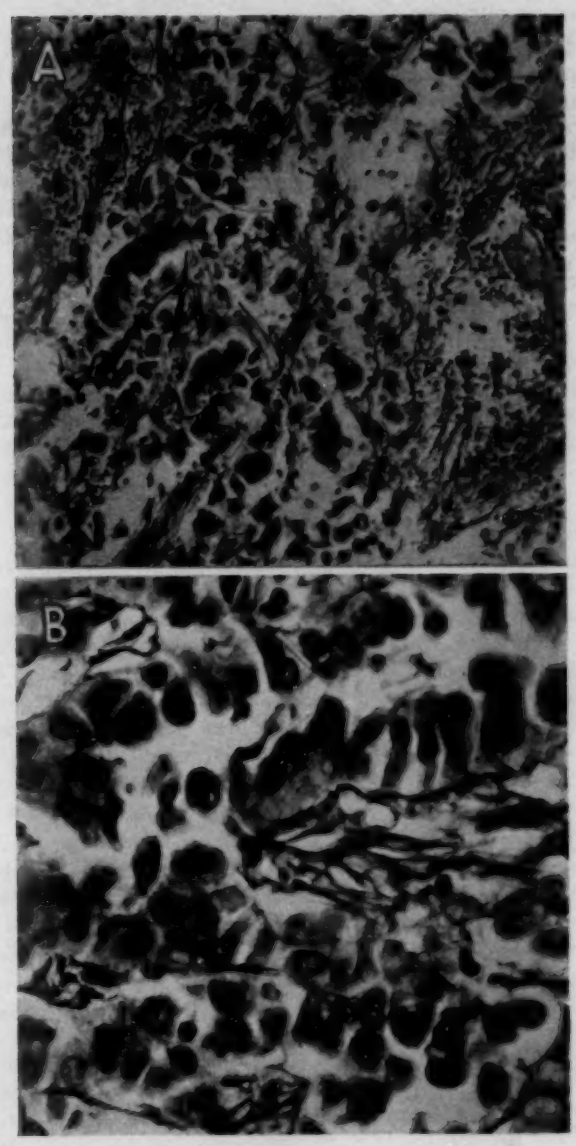

Fig. 3.—*A*, area from the mesentery stained for reticulum. The reticulum fibers are seen between groups of tumor cells, forming a fine supporting stroma. × 100. *B*, higher magnification of a section stained for reticulum, showing the fibers between the groups of tumor cells. It is seen that the individual tumor cells are not surrounded by the reticulum fibers and that these fibers do not extend into the tumor cells. × 400.

No metastases were reported by Miller and Wynn. Bender, cited by Miller and Wynn, reported no metastases in his case and assumed that the growth proves fatal before metastases occur. No metastases were present in our case.

SUMMARY

A case of mesothelioma (endothelioma) of the peritoneum is reported. Careful search failed to reveal any other tumor in the body. The similarity in structure between this neoplasm and carcinoma may be explained by the ability of the peritoneal cells to assume the role of epithelium and to produce epithelium-like structures. There were two types of connective tissue in the tumor. One was a reticulum-positive supporting structure; the other, reticulum negative, formed the stroma. No mucin was secreted by this tumor.

PULMONARY INFARCTION AND ATELECTASIS

Report of a Case Presenting Evidence of a Causal Relationship

BENJAMIN CASTLEMAN, M.D., BOSTON

In a recent paper by Fleischner, Hampton and Castleman [1] it was demonstrated that linear roentgen shadows are produced by platelike foci of atelectasis, healed pulmonary infarcts or interlobar pleuritis. Although mention was made of the fact that both atelectasis and infarction are prone to occur in areas of impaired ventilation and circulation, no definite evidence of any relation between the two was presented. A great deal, however, has been written about the relation of atelectasis to pneumonia, and many investigators, especially Coryllos,[2] have expressed the belief that both lobar and bronchopneumonia develop on preexisting atelectasis. In a paper on the circulation of the lung, Coryllos and Birnbaum [3] showed that the circulation and ventilation of the lung are "parallel functions"; when one is impaired, the other is also decreased. In the case to be presented here there is fairly good morphologic evidence that an infarct may develop in a preexisting atelectatic area.

REPORT OF CASE

A 56 year old woman entered the hospital for fecal incontinence and was found to have a tumor of the sigmoid with pelvic extension, diagnosed as carcinoma. A colostomy was performed, but following operation she gradually failed, and on the fourteenth postoperative day she died. A roentgenogram of the chest made on admission showed no evidence of disease. A postmortem teleroentgenogram (fig. 1 *A*), taken according to a technic previously described,[4] showed a definite linear horizontal shadow across the lower third of the lower lobe of the right lung. Since this shadow was not present on the preoperative roentgenogram taken nineteen days before, it was interpreted as probably a postoperative platelike atelectasis.[5]

Postmortem examination confirmed the diagnosis of carcinoma of the rectosigmoid with marked peritoneal extension and also with metastases to the liver. The base of the lower lobe of the right lung was adherent to the dome of the diaphragm by thin fibrous adhesive strands. The left pleural cavity was completely obliterated by old adhesions. No fluid was present on either side. Both lower lobes were slightly congested and subcrepitant. In the upper portions of the upper and lower lobes of the right lung there were characteristic brownish

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From the Department of Pathology and Bacteriology of the Massachusetts General Hospital.

1. Fleischner, F.; Hampton, A. O., and Castleman, B.: Am. J. Roentgenol. **46**:610, 1941.
2. Coryllos, P. N., and Birnbaum, G. L.: Arch. Surg. **16**:501, 1928.
3. Coryllos, P. N., and Birnbaum, G. L.: Arch. Surg. **19**:1346, 1929.
4. Hampton, A. O., and Castleman, B.: Am. J. Roentgenol. **43**:305, 1940.
5. Fleischner, F.: Wien. Arch. f. inn. Med. **28**:461, 1936.

red, well circumscribed infarcts, each measuring about 2.5 by 3 by 2 cm. Microscopically, these two infarcts showed practically no organization and were almost certainly of very recent origin.

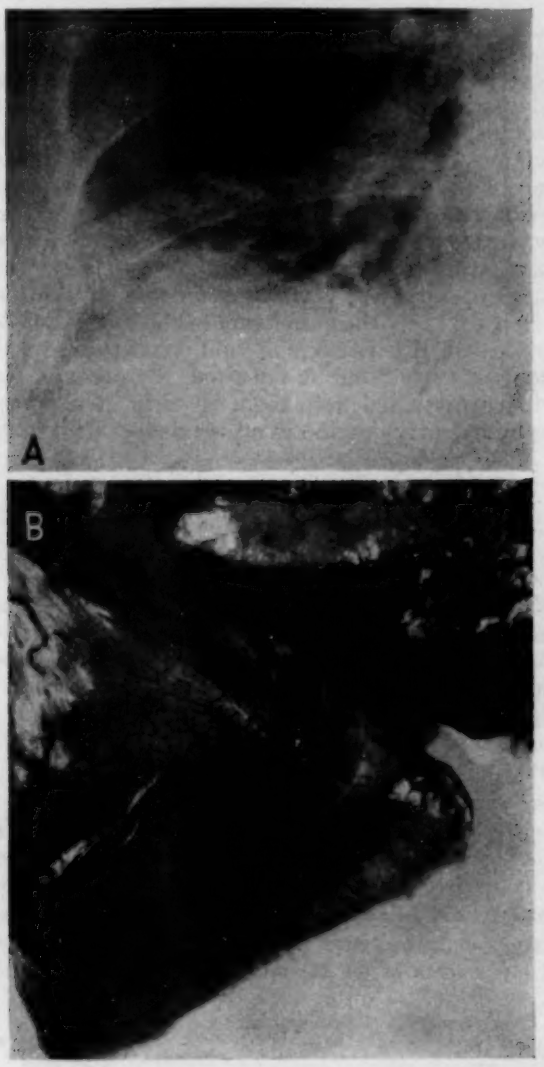

Fig. 1.—*A*, postmortem teleroentgenogram of the right lower lung field showing a homogeneous area of density placed against the lateral pleura. A roentgenogram taken nineteen days before did not show this abnormality. *B*, photograph of the lower half of the right lung, showing linear hemorrhagic sulci across the lower and middle lobes.

Across the lower third of the lower lobe of the right lung was a dark red depressed linear area extending from the anterolateral border horizontally across

the whole anterior surface of the lobe to the medial border and continuing along the medial surface in the region of the interlobar septum (fig. 1 *B*). This lesion was 7 cm. long and 0.2 to 0.4 cm. wide. It was deep red and quite firm, and when the lung was injected with 4 per cent solution of formaldehyde through the trachea, the distention of the parenchyma surrounding the linear area exaggerated the depressed cleftlike effect of the lesion. The fact that the latter could not be inflated does not rule out atelectasis, for in most of these cases it is almost impossible to overcome the surface tension of the contiguous alveolar walls. From the gross appearance at autopsy it was felt that the shape and the location of this lesion were characteristic of the platelike focus of atelectasis previously

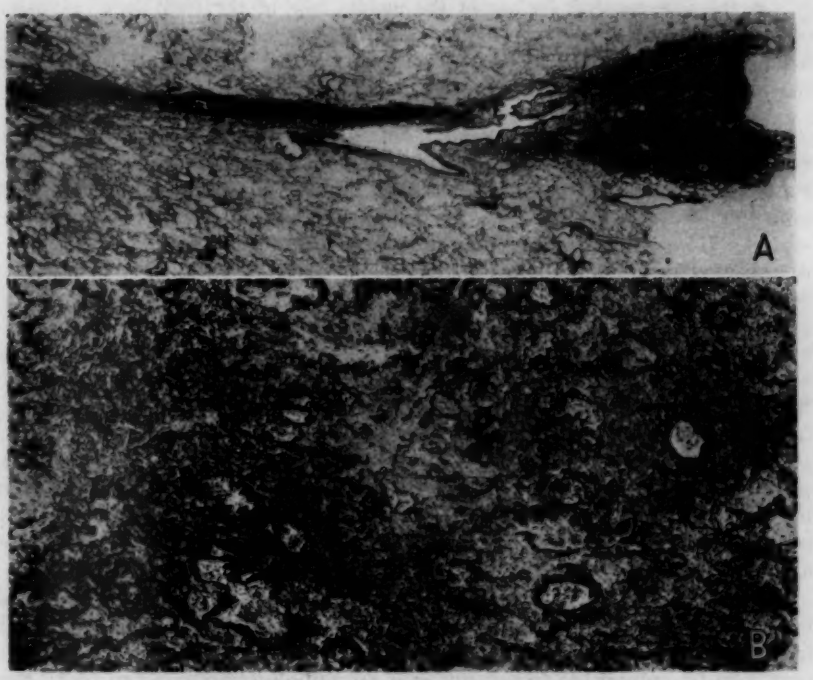

Fig. 2.—*A*, photomicrograph at very low magnification showing the shape of the lesion in the lower lobe (elastic tissue preparation). *B*, photomicrograph at higher magnification showing a recent infarction with destruction of elastic fibers (elastic tissue preparation).

described by Fleischner.[5] The red color and the apparent firmness of the linear lesion, however, were factors against that diagnosis, since atelectasis is usually blue or purple and in the linear form not firm. The other possibility was that it might be a healed infarct, but this could be ruled out by the fact that a healed infarct of this size must have been much larger in its acute stage, and it would have taken at least months to shrink down to its present size.[6] The

6. Castleman, B.: Arch. Path. **30**:130, 1940.

roentgenogram taken three weeks before death did not show any lesion. When sectioned vertically and perpendicularly to the pleura, the lesion extended into the parenchyma for 3 cm. as a thin rigid band, widest at the pleura and tapering off medially (fig. 2*A*). Microscopic examination of this lesion (fig. 2*B*) showed necrosis, hemorrhagic infiltration, deposition of hemosiderin and shadows of alveolar walls—observations characteristic of recent infarction. The infarction was most severe near the pleura. Definite recent thrombi were present in the pulmonary arteries, both within and just outside the infarct. Slight but definite organization of the peripheral margins of the infarct had already begun, which was evidence that the lesion was probably between one and two weeks old. The surrounding alveoli were partially atelectatic and in places filled with fluid.

On the anteromedial surface of the middle lobe of the right lung, extending obliquely downward and somewhat medially, was another very similar linear band, about 5 cm. long and 0.4 cm. wide, which was histologically the same as the one just described.

The important and somewhat intriguing feature of this case is to explain the genesis of the horizontal bandlike shapes of the recent infarcts in the lower and middle lobes of the right lung. In a discussion on the shape of pulmonary infarcts Hampton and Castleman [4] stated, "The shape of the infarct is dependent entirely upon the shape of the part of the lung it involves." The shape of the lesion in question is definitely that of platelike atelectasis, since there is no other recent process (it must be recent since a preoperative roentgenogram did not show it) that could involve a whole lobe over such a narrow area. The shape, the distribution and the size of the lesion are that of atelectasis, but the microscopic picture is that of infarction. Could the infarct have developed in a preexisting platelike focus of atelectasis? The presence of atelectasis around the lesion might be conceived as being part of the original atelectatic focus that had not become infarcted, but this evidence is not too valid since it might well have resulted from the pressure of the infarct on the adjacent alveoli, such as that seen around any area of consolidation.

The presence of two typical infarcts in this same lung in addition to the lesions in question is evidence that emboli were entering this lung, and any focus of impaired ventilation and circulation would most certainly be vulnerable to infarction if an embolus happened to reach that area. Whether the atelectatic focus was in any way a "drawing card" for the embolus is questionable, although Nissen [7] showed experimentally that emboli introduced into a peripheral vein will go regularly and without exception to that lung which has previously been collapsed. These experiments in rabbits were concerned with collapse of a whole lung, and it is doubtful whether a relatively small atelectatic focus could have a similar effect.

It has been fairly well established that bland pulmonary infarction does not occur in a normal lung, the usual prerequisite being pulmonary congestion. Although this case illustrates that infarction may develop in a preexisting focus of atelectasis—a condition in which both ventilation and circulation are impaired—it is not suggested that atelectasis

7. Nissen, R.: Arch. f. klin. Chir. **167**:567, 1931.

plays a role in the development of the usual infarct. Infarcts have often been found in atelectatic lobes, but a definite causal relation, although intimated, has never been proved. The unusual shape of the infarcts in the case presented affords more evidence of this relationship.

SUMMARY

A case is presented in which two linear recent pulmonary infarcts were observed at autopsy. This unusual shape for a recent infarct is given as evidence that the lesion developed on a preexisting platelike focus of atelectasis. The question of atelectasis as a predisposing factor for embolism and infarction is discussed.

Massachusetts General Hospital.

General Reviews

EFFECTS OF RADIATION ON NORMAL TISSUES

SHIELDS WARREN, M.D.
BOSTON

(Concluded from Page 139)

X. EFFECTS ON ORGANS OF SPECIAL SENSE

THE EYE

The natural apprehension lest the eye should be injured when radiant energy was directed to neighboring structures led to early consideration of its sensitivity. When roentgen rays and radium were first utilized, there were even claims that the blind could see by means of them. Animal experimentation has been of necessity the chief source of information as to the effects on the normal eye. There is little evidence dealing with the relation of the dose to the reaction of the human eye.

The severe destructive action of roentgen rays on the structures of the anterior segment of the eye, namely the conjunctiva, the cornea and the iris, in long exposures was described by Chalupecky in 1897. Although there is little difference in the sensitivity of these three structures, they react in this order. Conjunctivitis and keratitis are useful indications of the strength of the radiant energy, especially in the early reports, in which the factors of radiation are often not stated. The reaction is elicited by somewhat smaller doses than are required to produce dermatitis, and the latent period is generally shorter. This sensitivity of the outer parts of the eye is greatest in newborn animals and lessens with maturity. Conjunctivitis has been described after slight irradiation of the eye of a kitten (Belley), while in rabbits 2 to 4 weeks old conjunctivitis developed only after exposure to moderate to heavy radiation, depending on whether it was not or was filtered (Frogé). In the experiments of Birch-Hirschfeld (1904 a) the latent period seemed to depend more on the dose than on the age of the animal. The most marked pathologic changes were seen in the adult rabbit which had received 20 Holzknecht units in thirty minutes and was killed thirty days later. After a latent period of sixteen days, the following gross changes gradually appeared: mucopurulent conjunctivitis, interstitial

From the laboratories of pathology of the Harvard Cancer Commission, New England Deaconess Hospital and Pondville State Hospital for Cancer, and the department of pathology of Harvard Medical School.

keratitis, contraction of the pupil, and bleaching and hyperemia of the iris. The cell changes in the conjunctiva were marked in degree, especially in the region of the conjunctival fold, and irregular in distribution. There were desquamation of epithelium and different stages of degeneration with attempts at regeneration. The nuclei stained poorly; some of them were long and shaped like a biscuit or a dumbbell; atypical mitotic figures were present. Leukocytic infiltration appeared most marked around the hyperemic vessels in the region of the limbus, and the vascular endothelium was swollen. In the anterior layer of the cornea there was quite sharply outlined necrosis. The epithelium was in part desquamated, and in some places there was only a single layer of cells with elongate nuclei. At the periphery of the cornea, the epithelial cells were greatly swollen, their nuclei were irregular in shape and sometimes duplicated, and mitotic figures were more numerous. There was some general edema, as well as leukocytic infiltration. The reaction might last several months. One rabbit on whose eyelid an ebonite tube containing 20 mg. of radium bromide was allowed to remain for five hours showed, after a latent period of sixteen days, conjunctivitis and clouding of the cornea, which had not completely subsided eighty-nine days later. At this time the microscopic section showed moderate leukocytic infiltration of the conjunctiva and in the cornea moderate dilatation of interfibrillar spaces, irregularity of corneal corpuscles and questionable infiltration by leukocytes. The anterior chamber was filled with fibrinous exudate. Regeneration appeared first in the cornea of the rabbit, somewhat earlier than in the conjunctiva after 2 erythema doses, and might be seen for a long time afterward (Rohrschneider)—in 1 rabbit, two hundred and fifty-eight days after 1.5 erythema doses.

Jacoby irradiated adult rabbits with doses expressed as erythema doses for man. In one eye given 2.5 erythema doses in one hundred and fifteen minutes soon hyperemia of the conjunctiva developed, and later on abundant mucopurulent conjunctivitis. Epilation occurred in ten days and extensive radiodermatitis in three weeks. Twenty days after exposure the cornea showed flecks that finally merged to cover it completely. Dendritic erosions formed which subsided entirely after nine months. Another eye receiving 3 erythema doses showed epilation after two weeks and keratitis after three and a half weeks, subsiding in three and a half months. Five erythema doses given in three doses over a period of eleven weeks produced marked swelling of the lids and conjunctivas, abundant secretion and severe keratitis with ulceration, which continued undiminished five and a half months. At the end of this time microscopic examination revealed degenerative changes in the epithelium of the conjunctiva and the cornea, leukocytic infiltration,

especially around the vessels in the vicinity of the limbus, and swollen, vacuolated endothelium. Jacoby concluded that the epilation dose for the rabbit is approximately 2 erythema doses for man, and the injurious dose for the cornea is approximately 2.5 doses. The latent period for the reaction of the tunics was from two to five weeks.

The results of most investigators are in conflict with those of Rados and Schinz, who claimed 19 human erythema doses had no effect on rabbits' eyes, while 17 doses caused loss of hair from the eyelids after a latent period of three weeks and slight conjunctivitis and keratitis after four weeks. Twenty-one and 26 erythema doses produced total loss of skin from both eyelids and marked conjunctivitis and keratitis.

Cataract is the most important injury resulting from irradiation of the young or partly developed eye. It is produced by exposure to radiation (moderate or heavy, depending on the factors of time, filtration, and so on). The striking contrast in sensitivity between the lens of the young animal and that of the old one is explained by the fact that there is still active cellular proliferation in the young lens (Tribondeau and Belley). The equatorial and posterior cortical regions which are the most rapidly proliferating are said to be most affected by radiation (Bossuet). Lenticular opacity was found in a little over half of the fetuses whose mothers had been irradiated, even though in some instances the abdomen was shielded (von Hippel, 1907). Milroy observed the opposite in the chick embryo: a marked sensitivity of the retina as compared with the lens. Among the earliest reports are those of experiments on newborn kittens (Belley; Tribondeau and Belley; Tribondeau and Récamier). The superficial parts of the eye were found to be damaged by soft roentgen rays, while the lens was most affected by medium and hard rays. It was claimed that as little as five minutes' exposure to roentgen rays would cause a certain degree of opacity of the lens of very young kittens (Tribondeau) and almost complete vascular and granular degeneration of the lens of a 3 day old kitten resulted from the application of moderate unfiltered soft radiation (Tribondeau and Récamier [1]). Gross changes in the lens were noticed thirty-three days (Tribondeau and Belley) after treatment with soft and medium rays (Tribondeau and Récamier), moderate radiation (Belley; Tribondeau and Belley) giving microscopic evidence of injury fifteen to twenty-three days after exposure (Belley). The earliest abnormalities have been described as flattening and degeneration or complete disappearance of the lenticular epithelium on the anterior surface. Some hypoplastic thickening of the epithelium may occur. The lenticular fibers become enlarged and tortuous, owing to vacuolar and granular

1. The radiation treatment consisted of six exposures of ten minutes each at 10 cm. distance given at the rate of three a week.

change. The greatest effect was seen in the equatorial zone. Frogé produced cataracts in young rabbits with moderate to heavy radiation, filtered as well as unfiltered. The lenses became disproportionately small as the animals grew. Peter described cataracts in rabbits 5 to 10 months old, developing between forty-five and three hundred and twenty-five days after treatment, depending on the strength of the dose. The doses were 1 to 10 erythema doses (150-180 kilovolts; 1 erythema dose equals 600 r). Clouding of the lens occurred at ninety to two hundred and sixty days after exposure to from 160 to 640 milligram hours of radium. Microscopic examination showed vacuoles chiefly in the posterior part of the lens.

The lens of the adult rabbit is very resistant. No effect was observed up to six weeks after thirty to sixty minute exposures (Tribondeau and Lafargue). A similar resistance was observed by Jacoby after exposure of adult rabbits' eyes to 3 human erythema doses for sixteen minutes or 2.5 doses for one hundred and fifteen minutes, or 5 doses in three sessions over a period of eleven weeks. In the last instance the normal gross appearance was confirmed by microscopic examination five and a half months after the final treatment. Nor was the lens affected by placing 20 mg. of radium bromide on the eyelid for periods of two to six hours (Birch-Hirschfeld, 1904 b). Rados and Schinz described normal lenses in rabbits after 26 human erythema doses.

Bossuet made careful studies of the lenses of fetal, young and adult animals, using dogs, rabbits and guinea pigs, and concluded that a lens of any age may be injured by roentgen rays but that the effect is much greater in the immature lens. For his experiments on adult animals he used 2 rabbits and 2 guinea pigs. The rabbits were given forty-five minute exposures at 5 cm. distance, and the guinea pigs were given single exposures of forty-five and ninety minutes at 5 cm. The rabbits were killed two and thirty-one days afterward and the guinea pigs at ten and eleven day intervals. The rabbit killed after two days showed no change, but the other 3 animals had a similar reaction of the lens, consisting of degeneration of the capsular epithelium as well as cellular proliferation.

Meisner produced cataract in an adult rabbit by introducing 0.68 millicurie of radium emanation into the vitreous. Eight days later there was clouding of the vitreous, and twelve days later, slight blurring of the aqueous humor and fine precipitation on the anterior capsule of the lens. After sixteen days there was husklike opacity of the posterior capsule. The lens was totally opaque at the end of one month.

Thorium-x injected into the eyes of adult rabbits has produced suggestive but inconclusive results. Abelsdorff injected thorium-x into the aqueous of the eyes of adult rabbits in amounts equivalent to

the gamma rays emitted from 0.017 to 0.015 mg. of radium bromide. The experiment was controlled by an injection of inactive thorium. Forty-two animals were used. The lens generally remained clear, although cloudy areas in the posterior corticalis were sometimes seen several weeks after the injection. Stargardt injected slightly smaller doses of thorium-x into the eyes of 3 adult rabbits.[2] Complete opacity of the lens was found in the 2 animals killed one hundred and eighty-eight days after the injection, the first evidence of damage having appeared about the sixtieth day.

Early changes in the rabbit's lens as indicated by vacuolation were obtained with an epilation dose (1 erythema dose for a rabbit equals 1.8 erythema doses for a human being) by Rohrschneider (1929 c, d). This is less than the dose required to injure the cornea or the conjunctiva (2 erythema doses). He also stressed the length of the latent period and stated that perceptible changes in the lens were never found during the first three months after irradiation of the lens and that the latent period and the degree of opacity varied generally in relation to the quantity and the quality of the rays (Rohrschneider, 1929 a).

It is usually considered even more difficult to injure the retina than the lens, although London in 1906 claimed a high degree of sensitivity for the retina. He kept rabbits in a cage with 260 mg. of radium bromide for periods of some hours to seven days. Very severe injury of the skin of the ears and the back resulted.

Nor do the observations of Milroy accord with those of most workers. He exposed chick embryos to rays from 20 mg. of radium bromide, lead filtered, in an ordinary incubator for seven to eighteen days. Then the embryos were directly exposed for from one to three hours through an opening in the shell and the membrane. The eyes were then removed. In all the eyes, the retina showed degenerative changes, while other structures were hardly affected at all. All of the retinal cells were affected. The stratification of cell layers was lost as a result of the degeneration and the active regeneration. Pigment was partly extruded. Where destruction was greatest the ganglion cells were partly lost and some fibers of the optic nerve had degenerated. After seven days' exposure the degeneration was minimal. After fifteen to twenty days the rods and cones could still be identified, although they appeared to be destroyed almost as soon as they were formed. Considerable disintegration of the remaining mesoblastic cells was also noted. The lens and the muscle fibers were the least affected. The retinas of fetal

2. The quantities of solution injected into the anterior chambers and the vitreous were radioactively equivalent to 0.0045 mg. and 0.0025 mg. of radium bromide, for the anterior chambers, and 0.009 mg. for the vitreous.

rabbits were described by Pagenstecher as showing developmental abnormalities in the form of rosettes after three treatments with 1.25 erythema doses given one to two weeks after impregnation.

Tansley, Spear and Glücksmann gave a detailed account of the effect of radiation on the mitotic activity of the retina of the rat a few days after birth. The radiant energy was in the form of radium sulfate, 302.1 mg. being placed in a container and filtered with 5 mm. of platinum and mica. The intensity of the gamma rays on the surface of the skin was approximately 40 r per minute, or 300 Siefert units. The duration of the exposures varied from one-half minute to thirty minutes. Such radiation affected only those parts of the retina which were undifferentiated. The mitotic activity and the degenerative changes were described in great detail for long periods after the application of varied amounts of radiation. In 1 instance degeneration was noticed fifteen hours after a one minute exposure. Degeneration was first seen six hours after a two minute exposure and reached its maximum six hours later (i. e., twelve hours after exposure), at which time 12 per cent of the undifferentiated cells were degenerate.

An interesting abnormality of the retina, most marked in the region of the ora serrata, has been described in young animals (Tribondeau and Belley). It consists of an infolding of the anterior half or two thirds of the external layer of rods and cones. As a result of this process, external granular cells are isolated at the bottom of the folds. Structures resembling the rosettes of glioma may occur (Belley). This change is associated with, and probably due in part to, partial inhibition of the growth of the eye as a whole, a constant finding, and to difference in the growth rates of the layers of the retina at the time these were irradiated (Tribondeau and Belley). Even in the case of young animals very heavy radiation is necessary to produce changes at all clearcut in the retina and the nerve. Atrophy of the internal granular layer of the retina was described in the eye of a kitten which had been exposed to moderate radiation (sixty minutes in six sessions of ten minutes each in two weeks at 10 cm.) three days after birth (Tribondeau and Récamier). This change was observed at one month. The layer of nerve fibers was described as thickened, while the optic nerve was said to be normal.

Birch-Hirschfeld (1904 a) described partial destruction of the posterior pigment layer of the retina of an adult rabbit fifty-nine days after 10 to 12 Holzknecht units had been administered in twelve minutes at 10 cm. The only other change noticed in the eye of this animal was a slight haziness of the cornea.

Radium placed on the eye or within the eye will injure the retina and the nerve. Birch-Hirschfeld (1904 b) described rather vague degenerative changes in the retina and the ganglion cells of an adult rabbit, together with myelin degeneration of some of the fibers of the medullary rays (demonstrated by Marchi preparations). Twenty milligrams of radium bromide in an ebonite tube had been placed on the eyelid for two hours, and the microscopic examination showed a normal retina at the end of one hundred and twenty days. Eighty-nine days after a five hour exposure to the radium tube, the changes, though early, were slightly more definite. Some of the ganglion cells of the retina were vacuolated and the nuclei shriveled. A rather marked degeneration of myelin was seen in the optic nerve and the medullary rays. The same kind of change in the optic nerve, although possibly greater in degree, was found ninety-seven days after a six hour exposure to 20 mg. of radium bromide. The isolated retina of the adult rabbit first shows indications of injury from twenty to forty-five minutes after exposure (Birch-Hirschfeld, 1904 b). Tribondeau and Lafargue (1908) took issue with the findings of Birch-Hirschfeld (1904 b) and claimed that the changes which he described were within normal limits. Furthermore, two months after even larger doses they were unable to observe changes in the retina. Complete degeneration of the retina, degeneration of the medullary sheaths of the optic nerve, as well as opacity of the lens of an adult rabbit's eye, followed introduction into the vitreous of tubes containing 0.68 millicurie of radium emanation (Meisner).

Eichbaum injected a solution of radium chloride into the eye of a rabbit. One-half millicurie produced no changes. Four weeks after 1 millicurie had been given there were fibrinous and fibrous hyalitis, chorioretinitis and neuritis. One and five-tenths millicuries caused inflammation of the whole bulb, especially of the ciliary body, for one to four months after its administration.

Abelsdorff described disappearance of the layer of rods and cones, edema and loosening of the swollen layer of nerve fibers, as well as degeneration in the anterior portion of the optic nerve, after he had injected into the eye an amount of thorium-x equivalent to 0.1 mg. or more of radium bromide.

The pigment of the iris of the young animal is affected by moderate to heavy radiation and that of the adult by heavy radiation (Stargardt). The pigment granules become clumped in the chromatophores, and these disintegrate and finally disappear. The same type of change

appears in the chromatophores of the choroid after the latter has been heavily irradiated. Radiation sufficient to produce changes in the chromatophores will have produced more or less severe iritis with congestion, edema, petechial hemorrhages, posterior synechiae and later atrophy of the iris, including the ciliary muscle.

The most obvious expression of the inhibitory influence of radiation on the growing eye is the narrowing of the palpebral fissure. It is not entirely clear from the early reports how much radiation is necessary to retard growth (Belley). After exposure to moderate radiation, development does not stop altogether but continues at an apparently even, though much slower, rate.

Congenital abnormalities of the eye have been described by various authors. Three or more weeks after treatment of the pregnant mother, the animals are usually premature or at least stillborn and cadaverous. The radiation producing these effects has been moderate or heavy. Defects of the lids are a quite constant finding. Microphthalmos, coloboma (von Hippel, 1906) and hemorrhages into both chambers, as well as opacities of the lens, have been described (Bossuet; von Hippel, 1906, 1907). In most instances it is not possible to be sure which defects result primarily from the radiation directed to the structures of the eye and which are secondary effects.

Some of the discrepancies and negative findings (Roselli) reported may be laid to the making of observations too soon after the completion of radiation treatment.

THE EAR

The effects of radiation on the ear are at present but ill understood. This is natural since most of the diseases of the auditory apparatus itself are not susceptible to treatment by radiation and there are few lesions in the vicinity of the ear the treatment of which by radiation would lead to injury of the organ. Practically all of the early experiments are largely vitiated by the fact that extensive trauma was induced by the method of applying the radium or the radioactive substances; much of the reaction encountered was due probably to the implantation rather than to the effect of the radiation. Such are the early experiments of Ewald and Marx. These experiments present much detailed description of the behavior of the few animals and birds treated and of the lesions found. Chilow placed radon in glass in a tympanic cavity in each of 3 cats. There was marked hyperemia of the region; the cells of the organ of Corti were represented by a shapeless mass; the scalae were infiltrated by leukocytes, and there was an inflammatory picture through

most of the region. As Desjardins pointed out, these experiments were ill controlled.

Marked hyperemia of the middle and inner ear has been produced by roentgen radiation of unspecified amount (Thielemann). This hyperemia was so marked as to produce perilymphatic hemorrhage in the inner ear. Serous or seropurulent exudation developed in the middle ear.

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XI. EFFECTS ON ENDOCRINE GLANDS

The sketchy histologic data bearing on the sensitivity of the pituitary gland to radiation are in marked contrast to the great mass of relative clinical reports. Animal experimentation has given practically all the information at hand, and this is inadequate, especially because of the one-sided interest of most workers: Seldom have the factors of radiation and the physiologic and histologic observations been presented with equal clearness. In general the changes described are of a minor nature. Several studies on animals have demonstrated congestion, edema and "degeneration" of the anterior lobe after moderate and heavy irradiation with roentgen rays or radium. There is a general impression that the eosinophils are principally affected. They are often said to be decreased in number, although the cells are not usually counted. Degenerative changes in the pars nervosa and the pars intermedia have been described only after heavy irradiation.

Suppression of growth and sex function and slight histologic changes in the pituitary gland have been reported by several writers. Lawrence, Nelson and Wilson treated albino female rats 30 to 40 days old with roentgen rays (180 kilovolts) directed to the pituitary gland through a hole 1 cm. in diameter in a lead shield. It was found that 2,080 r in two doses a few days apart was the smallest amount of radiation which would affect, with any degree of certainty, either the general physical condition of the animal or the histologic structure of the pituitary gland. The changes in this gland were most consistent eighteen to twenty-two days after treatment. At later periods the gland appeared histologically normal. The functional effects closely resembled those following subtotal hypophysectomy: loss of body weight, hypoplasia of the pituitary gland, ovaries, thyroid gland and adrenal glands and loss of ovarian activity. In three reports changes in the pituitary gland were discussed which were so slight as to be questionable: Epifanio and Cola thought the growth of rabbits (both young and adult animals were used) was accelerated with 25 per cent of an erythema dose and inhibited with larger doses. Obvious suppression of growth and development was observed by Brunner in a kitten after 4 erythema doses given in one treatment and in dogs after 2.5 erythema doses given in four treatments three to four days apart, and by Fraenkel and Geller in bunnies after 75 to 50 x units. Rather more definite histologic changes were described by Podljaschuk (1927); degeneration and atrophy were present in the

anterior lobes of dogs fifty-eight days after a 130 per cent erythema dose and glial proliferation in the posterior lobes seventy-two days after this treatment.

Other workers using comparable doses found no change in the pituitary gland to correspond with the physiologic changes.

Fehr gave sexually mature rabbits massive doses—3,300 r to 10,160 r (180 kilovolts). The dose reaching the pituitary gland was estimated as 80 per cent of the dose reaching the skin. Massive doses produced loss of body weight and atrophy of the genitalia, whereas, if the doses were fractionated, the animals continued to gain in weight. The pituitary glands were examined four to six weeks after irradiation. Martinalli reported loss of body weight and pathologic changes in the ovaries and uteri of mature rabbits resulting from exposure to radiation, although the pituitary glands of these animals remained normal.

Contradictions found from one report to another result partly from difference in the interpretation of observations. The findings of Ghilarducci are especially hard to reconcile with other reports. Irradiation of young rabbits produced death, with complete destruction of the anterior lobe of the pituitary gland and skeletal deformity, but the ovaries and other endocrine organs were normal.

In some reports the pertinent data are chiefly histologic. Thus Cannavo and Beninato described "marked change" in the pituitary glands of rabbits dead of cachexia fifteen to twenty days after the administration of 700 to 1,000 r in three doses. This "change" consisted of swelling of eosinophils, without reduction in their number, and reduction of chromophobes. The pituitary glands of adult dogs showed varying degrees of atrophy and degeneration of the anterior lobe after 85 to 90 erythema doses (Podljaschuk, 1928). A reduction in the number of eosinophils in the pituitary glands of guinea pigs was described by Franck thirty-eight to seventy-seven days after large doses of roentgen rays. The metabolism of these animals was studied by Okkels and Krogh in connection with changes in other endocrine glands. The results were not outstanding.

In some instances, irradiation of the pituitary region has been without effect. Del Buono, using adult dogs, found no alterations in the pituitary gland or other organs six months to a year after nearly an erythema dose had been directed to the pituitary region. Kotz, Elward and Parker treated rabbits with a single dose of radiation (200 kilovolts) in amounts which were tremendous as compared with therapeutic doses. There was no demonstrable change in the weight of any animal or in the size or

structure of the gland three to seven weeks after treatment. The normal ratio of the eosinophils, basophils and chromophobes of the anterior lobe was maintained and there was no degeneration.

Selle, Westra and Johnson (1935, 1938) studied the physiologic effects of irradiation of the pituitary gland on 7 depancreatized dogs. The treatments were given several weeks after the dogs had been operated on; 2 dogs received a single treatment, and 4 dogs had two treatments three to four weeks apart. The estimated doses reaching the pituitary gland at 140 kilovolts were 1,500 r and 2,025 r for a single dose and 2,360, 2,045, 2,165 and 3,170 r for a total of two doses. The two treatments were given about three weeks apart, and the animals were killed three to four weeks after the single treatment or after the last treatment. Five animals (1 dog was not examined) showed some sort of mild change in the pars tuberalis such as numerous eosinophils after 2,045 r, edema after 2,165 r or "slight degeneration" after 2,025 r and 1,500 r, and "marked degeneration" after 3,170 r. Degeneration in the pars nervosa and the pars intermedia was slight after 2,165 r and marked after 3,170 r. Apparently there was no actual necrosis.

The only reference to the histologic effect of radiation on the human pituitary gland is a remark by Friedman that he had seen a normal pituitary gland after a total depth dose of 20,000 r given over a period of several years.

The clinical effects which have been described after roentgen treatment of the diseased pituitary gland as in the case reported by Cushing, are not elucidated by the studies on the normal animal's pituitary gland.

After due allowance has been made for the rough methods of estimating injury of cells, the difference in size of laboratory animals, methods of treatment, and so on, it seems that only heavy radiation has a very disturbing effect on the normal pituitary gland and there is little chance that therapeutic radiation affects it at all. It is possible that roentgen rays may have an entirely different effect on the diseased gland.

THE PINEAL GLAND

My associates and I have found no data bearing on the effects of radiation when this is directed at the pineal gland.

THYROID AND PARATHYROID GLANDS

A large amount of data has been made available as to the effects of radiation on tumors of the thyroid gland and on toxic goiter. This review, however, is restricted to the effect of radiation on normal tissues, and the data in this connection are relatively few. The thyroid gland may be considered fairly resistant (Walters, Anson and Ivy, 1932).

The earliest observation of an irradiated thyroid gland is that of Murray, who noted diffuse growth of the interalveolar connective tissue, possibly as a result of the parenchymal atrophy following irradiation.

Many of the early experiments were inconclusive but tended to emphasize the resistance of the thyroid gland (Krause and Ziegler). However, in many animals the skin over the thyroid gland showed no change; so the irradiation of the gland must have been slight.

By direct implantation of radium, Bower and Clark, using 12.5 milligram radium needles, produced necrotic lesions in dogs after doses varying from 25 to 169 milligram hours. Up to the third week a yellowish zone of necrosis bordered by a narrow reddish hemorrhagic band surrounded the site of the needle. Microscopically, the picture was one of necrosis and hemorrhage; the central material was completely amorphous but merged into a peripheral zone of necrosis resembling that seen in an early infarct with shadowy outlines of preexisting structures. In the outer border of this zone were remnants of fragmented nuclei, lymphocytes, a few plasma cells and occasional polymorphonuclear leukocytes. Surrounding this was a zone of hemorrhage showing only slight degenerative histologic changes, such as swollen cuboidal epithelium with deeply stained nuclei and scanty colloid. Beyond this, the thyroid tissue was essentially normal. After about three weeks, organization and healing began as a result of peripheral fibroblastic proliferation. There was some phagocytosis of the necrotic material, and occasionally calcification occurred.

The thyroid gland of the rabbit, although physiologically labile, requires very heavy radiation to affect it (Coulaud). Heavy roentgen radiation produced slight alteration in follicular size and some nuclear degeneration but no fibrosis. Guinea pigs were exposed to 50 to 400 milligram hours of radium radiation at a distance of 1 cm., and their thyroid glands were removed from one to fifty-eight days later (Eckert, Probstein and Galinson). The glands of the treated animals could not be told from those of the control group. After three fourths of their thyroid tissue had been removed, the guinea pigs were exposed to 100 to 150 milligram hours of radium and killed from one to fifty-eight days later. These glands showed some depletion of colloid and some lymphocytic infiltration.

The claim has been made (Florentin and Watrin) that roentgen treatment can produce in guinea pigs a hyperplasia similar to that seen in pregnancy, with increase in size of some of the cells and some mitotic activity. The nuclei described were not unlike those sometimes seen in slight spontaneous hyperplasia.

Pfeiffer failed to find changes in the thyroid glands of normal dogs after these had been given roentgen radiation but did obtain shrinkage of the thyroid glands in over half the patients with goiter who had been treated with doses large enough to redden or blister the overlying skin. Similarly, Rave observed no histologic changes in the thyroid glands of rabbits with doses of from 12 to 36 Kienböck units (about 1 to 3 erythema doses); in patients with goiter he found diminution of lymphoid tissue but no change in epithelium or colloid.

Young rats are more susceptible than adult rats, 1,000 r shifting the epithelium from cuboidal to flat and increasing the colloid storage (Viethen).

The claim is made by Takayama that he produced changes in the Golgi apparatus of the thyroid parenchymal cell with 35 r. This I cannot accept.

Heavy roentgen radiation produced hyperemia of the thyroid gland, followed by cellular degeneration and finally necrosis (Redaelli).

Slight hyperemia progressing to slight nuclear degeneration as the dose was increased to 1 erythema dose was observed by Tsuzuki.

Walters, Anson and Ivy (1931) irradiated dogs at 170 kilovolts, giving four doses of 380 r each from seven to fourteen days apart. In man three such doses on successive days resulted in a second or third degree burn. The dogs received from one to three series delivered to the region of the thyroid gland at varying intervals of time. Between some series, portions of the thyroid gland were removed. In some of the more heavily irradiated animals, thickening of the capsule had occurred. The irradiated animals showed a slightly greater tendency toward hyperplasia than normal dogs. The only change noted histologically was the capsular thickening, which developed only in those animals with burns and edema of the skin. This offers marked proof of the resistance of the gland.

Zimnitsky, Baskina and Devirz irradiated 15 male rabbits at 140 kilovolts giving 550 r daily in from three to ten daily doses. The animals were killed from one day to forty days after treatment. The thyroid glands of some of them showed slight hyperemia; those of others, slight vacuolation of the cytoplasm; with larger doses there was some coarsening of the mitochondria. Up to a total of 10 erythema doses the structure of the thyroid gland was practically normal. Possibly, there was a slightly higher epithelium lining the follicles than in the normal gland. Isolated cells or cell groups were sometimes affected without change in the parenchyma as a whole. The roentgen rays may stop for a time the production of colloid, so that it may

largely disappear. There were some vascular and connective tissue changes, with a slight increase in the stroma after some time had passed.

Almost no information is available as to radiation effects on the parathyroid glands. Bower and Clark reported no changes in their dogs, but probably the radium needles in the thyroid gland were not close enough to have an effect. According to Zimmern and Battez, sufficiently heavy radiation to produce atrophy of the thyroid gland caused atrophy of the parathyroid glands as well. In the experiments of Walters, Anson and Ivy, after heavy irradiation 1 dog showed hyperplasia of the parathyroid glands, with some fibrosis and capsular thickening. In 2 other dogs the radiation prevented compensatory hyperplasia following removal of two glands.

THE THYMUS GLAND

For the effects of radiation on the thymus gland, see chapter III.

THE ISLANDS OF LANGERHANS

For the effects of radiation on the islands of Langerhans, see chapter IV.

THE ADRENAL GLANDS

Interest in radiation effects on the adrenal glands has come about from two sources: first, the desire to influence various types of pathologic change associated with hyperfunction or hypofunction of these glands, and, second, concern as to the changes that might be induced in an adrenal gland as a result of its exposure to incidental radiation in the course of the treatment of lesions in adjacent organs. Thus a number of the earlier reports, practically all of which are unsatisfactory, deal with the roentgen treatment of Addison's disease. An example of the inadequacy of much of this early accumulation of data is Wiesner's case of Addison's disease, in which improvement followed roentgen treatment although later the patient failed and died. Very similar is the case reported by Golubinin in which a 27 year old man with Addison's disease was markedly improved after fifty small exposures to roentgen rays in seventy days. No follow-up of this case is given.

Some of the early experiments relating to functional aspects seem rather fanciful, as that of David and Hirsch, who claimed that irradiation of isolated adrenal glands with 1 erythema dose resulted in a decrease of the content of epinephrine and that irradiation with 25 per cent of an erythema dose increased the production of epinephrine. The data as presented do not inspire confidence in the results.

An early attempt at hormonal assay of irradiated adrenal gland tissue was carried out by Eisler and Hirsch, who irradiated the whole bodies of rats with 150 to 200 Kienböck units in eight to ten days. An extract of the irradiated adrenal glands was injected into the blood stream of 9 rabbits, and in 4 there was a transient elevation of the blood pressure greater than that produced by injection of an extract from nonirradiated adrenal gland.

It is unusual to find first rate evidence of injury of the adrenal glands as a result of therapy. But that in the case of Smithies is probably good. A healthy 58 year old man received extremely heavy radiation for a supposed tumor of the dorsolumbar part of the spinal column. A month later symptoms of Addison's disease developed, and the patient died soon afterward. There was no microscopic study. On the other hand, it is hard to believe that 30 per cent of an erythema dose as measured to the adrenal glands of a 58 year old woman could have resulted in almost complete fibrosis of the cortex six weeks later, as described by Stephan.

An adequate critical review of the clinical and physiologic aspects of irradiation of the adrenal glands has been made by Desjardins. The little concrete information which one finds is based on histologic observations in animal experiments. Early investigators (Harvey; Strauss; von Decastello) found congestion or hemorrhage or both in mice, guinea pigs and rabbits, which were usually subjected to fairly heavy doses of soft roentgen rays. Atrophy of the adrenal glands in mice as a result of exposure to radiation is an unusual finding which has not been confirmed (von Decastello). Destruction of adrenal tissue has been produced by direct implantation of radium (Wislocki and Crowe). Lacassagne and Samssonow embedded unfiltered tubes containing 4.5 to 10 millicuries in the adrenal glands of rabbits. At death, from five to thirty-one days afterward, there was complete necrosis of these glands except in 1 instance in which a small amount of the glomerular layer persisted. Smaller doses of platinum-filtered emanation destroyed the medulla, leaving some of the cortex intact.

More detailed experiments on the effect of roentgen rays have brought out three points of interest: (1) a certain degree of sensitivity of the cortex; (2) a high degree of resistance of the medulla; (3) the difficulty of distinguishing minimal changes, especially in the cortex.

The great variability in the appearance of the normal cortical cells, chiefly with regard to the lipoid content of the cytoplasm, adds an element of uncertainty regarding the minor changes seen in the irradiated gland. The descriptive term "degeneration" usually applies to the amount of cytoplasmic fat and the degree of pyknosis. Alterations of lipoid content, usually a decrease, have been described frequently.

However, after well controlled studies, Engelstad and Torgersen were unable to form any definite opinion on this score. Tsuzuki regarded reduction of lipoid as the earliest change. This appeared immediately after 32 per cent of an erythema dose [1] had been given and lasted for at least ninety-six hours. Twenty-four hours later there was degeneration of the medullary cords. Similar reduction of fat was noted after the administration of 48 per cent and 64 per cent of an erythema dose. Animals exposed to radiation continuously until death showed reduction of fat after sixty minutes (32 per cent of an erythema dose) and hyperemia and "degeneration" of medullary cords after ninety minutes (48 per cent of an erythema dose). In general at death the irradiated organ was slightly enlarged and often hyperemic. Holfelder and Peiper noticed diminution of lipoid in the adrenal glands of guinea pigs after small doses around 80 per cent of an erythema dose. There was slight vacuolation of the cells, as well as irregularity in the size and the staining properities of the nuclei. Some irregularity in the distribution of reticular pigment was seen. All of the changes were present in reticular and fascicular zones; the glomerular zone remained normal. One animal that received 160 per cent of an erythema dose on each of two fields died in four days without macroscopic or microscopic changes in the irradiated gland.

More severe changes follow exposure to heavy radiation but bring out more clearly the absence of any close correspondence between cytologic change and dosage. These changes reflect the difference in sensitivity of animals of the same species and age (Holfelder and Peiper) and a lack of uniformity of reaction from cell to cell of a given organ (Engelstad; Engelstad and Torgersen). The variability of changes due to radiation cannot be reiterated too often. Engelstad was unable to demonstrate any appreciable changes in the adrenal glands of rabbits with doses under 1,500 r, a dose which approaches the skin's tolerance, although there was slight hyperemia of both cortex and medulla twenty-eight days after 1,000 r had been given. The first week after the application of 1,500 r, the adrenal glands appeared normal, but later mild degenerative changes appeared. Larger doses (3,000 r) caused pronounced degeneration of cortical cells initiated fifteen days later and reaching a maximum in the second month. Hyperemia and lymphocytic infiltration of varying intensity were associated with the degeneration. In 1 instance small abscesses formed. The injury seemed to be greatest in the zona fasciculata and the zona reticularis and no definite changes were found in the medulla. Slight fibrosis was present forty-six days after the administration of 3,000 r in a single dose.

1. All the doses given are those calculated for the adrenal glands.

In supplementary experiments on a large number of rabbits, the most constant changes were found two to six months after irradiation of the organs. Treatment consisted in the administration of 2,200 r or 2,500 r in one sitting (4 by 6 cm. field, with some animals being irradiated by anterior and some by posterior routes; 175 kilovolts; 20 cm. distance). The animals were killed mechanically from four to six months later, with the exception of 6 that died fourteen days to six months after treatment. Four degrees of change were described, not all of which are entirely clearcut to the reader. The earliest reaction consisted of hyperemia. The mildest cell change admittedly could not always be distinguished from variations of the normal gland. Clear-cut degeneration accompanied by more or less pronounced hyperemia was first seen six days after irradiation with 2,200 to 2,500 r, evidenced by more abundant cytoplasm and poorly stained nuclei with a dark perinuclear zone. The greatest and most constant injury, consisting of marked dissolution of cells and loss of structure, occurred at the end of three months. In some instances it was seen as late as six months. The glomerular zone was less affected than the others, and there was no bona fide change in the medulla.

Cortical necrosis of rabbits' adrenal glands, examined within forty-six days after the last treatment, followed saturation of the organs at various doses for a week or a week and a half by the method of Kingery. The irradiation of the animals was sufficiently severe to cause death of some from intestinal disturbances (Grabfield and Squier).

Rogers and Martin exposed adrenal glands of 16 dogs directly to unfiltered roentgen rays. Graded doses of from 3.5 to 32 erythema doses were given. The capsule was described as thickened in each instance. Ten erythema doses produced some extension of fibrous tissue into the cortex. This was marked in the cortex after 14 to 19 erythema doses, but only slight in the medulla. One dog died twenty-four days after receiving 32 erythema doses and showed gross edema, complete disorganization of the gland and thrombosis of the lumbo-adrenal vein.

While the cortex showed evidence of injury with moderate to heavy radiation, its zones vary in response. The glomerular is usually described as less sensitive than the reticular or the fascicular zone (Engelstad; Holfelder and Peiper). The glomerular layer of the rabbit shows the least changes with severe cortical damage (Engelstad and Torgersen). Cottenot, Mulon and Zimmern described complete destruction of the reticular and fascicular zones after 54 Holzknecht units of medium quality roentgen rays had been administered in five treatments to each adrenal gland of 1 dog, and in changes in the glomerular layer suggesting compensatory proliferation. The examination was made quite

late post mortem. However, Rogers and Martin found the opposite to be true after the use of heavy unfiltered radiation; i. e., the fascicular zone was less affected than the other zones and maintained its histologic structure after being irradiated with 32 erythema doses, though other cells had disappeared. This zone alone was viable in the adrenal gland of 1 rabbit that died thirty-one days after 6.5 millicuries of unfiltered radium had been inserted in the organ (Lacassagne and Samssonow).

Although preservation of the medullary structure in the presence of clearcut degeneration or necrosis of the cortex has been described by several workers (Engelstad), severe fibrosis of the gland, except for small islands of normal cortical cells, was found twenty-six days after heavy radiation had been applied directly to the gland (Martin, Rogers and Fisher). Tsuzuki reported that degeneration of "medullary cells" occurred twenty-four hours after the administration of 32 per cent of an erythema dose while the cortical cells were little changed.

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XII. EFFECTS ON BONE, CARTILAGE AND TEETH

OLIVE GATES, M.D.

BONE

In considering the histologic effects of radiation on bone it may be helpful to keep in mind certain facts regarding the structure of bone. Bone as a product of cell activity formed to give support and rigidity

is largely an intercellular substance of high mineral content. Minor alterations of the physical or the chemical structure of acellular tissue may produce little visible change. The difference between viable and nonviable bone may be only a slight variation in staining reaction and in the appearance of osteocytes unless disruptive forces, such as trauma or infection, intervene. For this reason effects of radiation on bone are less easily estimated. Moreover, the actual intensity of a given dose of radiation may be greater in bone than in other tissues because of the secondary radiations from the calcium.

The effect of radiation on growing, repairing and formed bone will be discussed as observed in experimental work on animals and as incidental to therapy.

Data from Experiments on Animals.—Retardation or cessation of growth of bone in young animals is a quite constant effect of radiation, provided the dose is above the threshold of tolerance. Although the reports in the literature often give seemingly inconsistent data, these may be reconciled in part by keeping in mind that variability is characteristic of all biologic reactions and that the reaction of living tissue is conditioned by its age.[1] The damaging doses of roentgen rays for bones of an infant and a child have been roughly estimated as 25 per cent and 50 per cent, respectively, of the erythema dose for an adult (Flaskamp). Difference in capacity for growth at different ages may explain variations in reaction when two bones of the same animal, as tibia and femur, are exposed simultaneously (Dahl; Engel). Generally, the degree of retardation is in direct relation to the dose up to the point of maximal response (2,400 to 3,000 r of high voltage rays, according to Gall and co-workers; 360 milligram hours, according to Engel), but the degree of retardation may be greater than the proportional increment of dose (Brooks and Hillstrom). Fractionation of the total dose and protraction of the interval between exposures greatly lessen the effect on growth (Bisgard and Hunt). As would be expected from observations on other tissues, equal doses of high voltage and supervoltage roentgen rays have been shown to produce identical effects except for slight difference in the timing of the reaction (Gall and co-workers).

Definite inhibition of growth [2a] has been observed in newborn and very young animals of different species,[2b] and this effect may be seen

1. Baunach. Engel. Iselin. Scott.

2. (*a*) Moderate amounts of roentgen radiation (750 to 1,500 r at 200 kilovolts) given in a single dose through a portal 5 mm. in diameter over the distal femur to young rats produce slowing of the longitudinal and the transverse growth. The effect depends chiefly on the age of the animal. No changes in bone salts were brought about. (Hinkel, C. C.: Am. J. Roentgenol. **47**:439, 1942.) (*b*) Försterling. Perthes. Tribondeau and Récamier.

after as little as 18 per cent (Marie and co-workers) or 40 per cent (Brooks and Hillstrom) of an erythema dose given in a single treatment. After 2,600 r given in one exposure to young rabbits, growth was barely appreciable by roentgenograms for several weeks (Regen and Wilkins, 1936a). Brooks and Hillstrom, using rabbits, compared growth curves of normal bones with those of bones given a single exposure of 1 erythema dose and found an inhibitory effect in a change of rate but not of duration of growth. Complete cessation of growth was simultaneous in the irradiated and in the corresponding normal bone. The authors suggested that the destruction of cells with greater growth capacity and shorter life would diminish the rate without necessarily shortening the period of growth. Bisgard and Hunt, however, described a slower but constant rate of growth which stopped abruptly just before growth had been completed in the normal bone.

Bones of animals stunted by 600 r showed a decrease in phosphatase activity, most marked in the metaphysis, beginning a few days after exposure, reaching a minimum value within one to three weeks and recovering after four to five weeks (Wilkins and Regen, 1934a). This was not associated with change in the electrolytes (Wilkins and Regen, 1934b).

The final effect of radiation on developing bone depends on the number of cells damaged beyond recovery. The contour of irradiated growing bone is usually close to normal (Brooks and Hillstrom). Where there is deformity, it is due to two factors: muscle strain and greater injury to one part of the bone than to the rest.

No stimulation of growth of bone—i. e., in the gross sense of increase in size—was noted in young rabbits after very small doses, 2 to 5 per cent of an erythema dose (Brooks and Hillstrom), but Baunach, using 25 per cent of an erythema dose, thought there was a suggestion of greater growth.

The two histologic changes most frequently described in dwarfed bones of animals as an effect of radiation are early alteration of cartilage cells and disorientation of endochondral ossification. The earliest changes in epiphysial cartilage cells, such as swelling, pyknosis and loss of columnar pattern, were observed two weeks after 50 per cent of an erythema dose in rabbits (Bisgard and Hunt) and one to two weeks after 600 r (200 kilovolts) in rats (Gall and co-workers).

The outer primitive cartilage cells never show more than slight change, but there seems to be some inhibition of their development into chondroblasts (Gall and co-workers). The least differentiated cartilage cells next to this outermost layer of primitive "mother" cells are the first to be affected (Gall and co-workers). Increasing amounts of radiation affect still better differentiated cells, and the columnar pattern becomes quite disorganized. A simultaneous change in the matrix

occurs which has been described as a coarsening and later a loss of fibrils. The same effect was seen in the epiphysial cartilage of the right femur and the right tibia (although most marked in that of the femur) two weeks after a 5 mg. radium needle was implanted subcutaneously for three days on the lateral side of the proximal end of the right tibia (Engel). After 1 erythema dose given in a single exposure to rabbits, some cartilage cells were severely damaged and disappeared, while others remained normal (Brooks and Hillstrom). Irreparable arrest of the growth of cartilage was produced after 550 r to 880 r had been given in fractionated doses (Baunach). Focal necrosis of cartilage has been observed in animals irradiated with 1,650 r (fractionated) (Baunach).

Some degree of recovery of cartilage has been described even after severe damage, and occasionally a large portion has regained its normal state. Usually the proliferating cells have lost their polarity, and even though they have the appearance of normal cells, there is no effective growth (Gall and co-workers). Bisgard and Hunt described complete loss of pattern with extreme degenerative changes in cartilage cells some days after 1,540 r was administered in a single dose to long bones of rabbits 4 weeks old, but on the thirty-sixth day columnar structure and normal-appearing cartilage cells were again recognizable. The final result is irregularly thickened and often partly ossified cartilage.

The exact mechanism of the interference with endochondral ossification has not been clearly described. This seems to be due to difficulty in estimating the pathologic changes in osteoblasts. Dahl described suspension of vascular invasion and of ossification of the cartilage. Gall and co-workers, using rats, observed increased osteoblastic activity accompanying the earliest change in chondroblasts one to two weeks after exposure of bone to 600 r. Even though the osteoblasts appeared to have been injured, there was slight excess of bone deposit on the primary cartilaginous trabeculae. The ossification of these primary trabeculae increased with higher doses; they became extremely thick and, in the absence of the propelling force of the growing cartilage, formed a bony plate at the epiphysial line. The bone thus formed was abnormally brittle (Gall and co-workers).

On the other hand, Dahl described premature calcification and inhibition of endochondral and periosteal ossification soon after epidermicidal doses were directed to the adjoining ends of the tibia and the femur in rats 12 to 18 days old. Calcification appeared first in the metaphysis on the third day and in the epiphysis on the fourth day. In the femur the endochondral ossification of the metaphysis was completely suspended by the fourth day, but in the tibia it continued sluggishly. Similarly, periosteal ossification ceased almost immediately in the femur but only after four to eight days in the tibia. Endochondral

ossification and periosteal ossification are thus of the same radiosensitivity (Dahl). Resorption of the diaphysis occurred in the tibia in the course of three to six weeks. The inhibition of ossification disturbed the normal balance of new bone formation and resorption. The continued resorption, calcification and devascularization of marrow and periosteum produced bone which was partly devitalized, partly sclerotic and heavily calcified (Dahl).

In summary, studies of the epiphyses of animals after the administration of roentgen rays have demonstrated high sensitivity of cartilage cells and less obvious sensitivity of osteoblasts. There is loss of polarity of cartilage cells and disorganization of endochondral ossification. Calcification is disturbed. The normal rhythm of bone formation and resorption is lost. Histologic evidence of injury is found in both chondroblasts and osteoblasts, and the intercellular matrix of each is affected.

Evidence regarding the effect of radiation on healing bone is likewise lacking in uniformity, but it is probable that this is due to difference of methods as well as to variation in interpretation. Cluzet, using moderate doses of low voltage roentgen rays, found that in rabbits and dogs there was marked delay in the formation of callus whether radiation was given before or after the bone was broken and even though the dose was insufficient to produce dermatitis. But Fukase has been cited to the effect that a dose of 400 r stimulated the formation of callus. Simon, using adult rabbits, transplanted segments of bone 1 cm. long from one bone to another and studied the effect of roentgen rays on absorption and union. Roentgenologic, gross and histologic observations indicated that the rays accelerated absorption of the transplant and also the process of union, which nevertheless followed normal lines. He expressed the belief that this was not due to stimulation of growth but was the result of rather greater injury to the transplant than to the adjacent tissues.

Brooks and Hillstrom made equal defects in the shafts of the right and left ulnas of young rabbits by subperiosteal resection and then submitted the right foreleg of each to an erythema dose either immediately after operation or on the third and sixteenth postoperative days. Although there was no perceptible difference in the amount of new bone formed or in the duration or the completeness of repair, the growth of the irradiated ulna was inhibited in each case.

Albee, using young adult to middle-aged rabbits, claimed no difference in the time or the completeness of union or in bone growth between irradiated and nonirradiated fractures or resected bone defects, even after massive doses had been given in several exposures five days apart.

On the other hand, Regen and Wilkins (1936b) found marked delay in the healing of fractures in ulnas of adult rabbits as compared with

the control. Animals were exposed to roentgen rays twice before fracture; the first dose was 1,112 r, and the second, two weeks later and one day before operation, was 622 r. Preliminary experiments had shown that phosphatase activity was consistently low in normal bones for four to five weeks after such treatment. The roentgen treatment given before fracture inhibited the rise of phosphatase activity which normally accompanies healing of bone. The callus was small in amount, and rigidity was delayed (Regen and Wilkins, 1936b).

Bade and Küntscher described moderate changes in bone cells of dogs after resection of a segment of radius and exposure to heavy radiation. Fusion of the cut ends of the radius did not occur as it did in the nonirradiated resected bones.

There are few experimental data which tell us what radiant energy does to adult normal bone. Nageotte suggested that although the gross appearance of dead bone may not be distinctive, degenerative changes in bone cells would be observed more frequently if looked for properly. On the other hand, Flaskamp expressed the opinion that healthy bone of adults is resistant to radiation.

Absorption and decalcification, with or without spontaneous fracture, have been seen in animals after exposure to heavy radiation,[3] implantation of radon seed (0.16 millicurie) (Franseen and co-workers) or as a result of chronic radium poisoning.[4]

Because of the tendency of heavy metals to be deposited in bone, radium poisoning has been especially productive of information in relation to the effect of radiant energy on bone.

Thomas and Bruner, studying chronic radium poisoning in rats, found both destruction of bone and increased growth. From 40 to 60 micrograms of radium chloride was given by subcutaneous injection during periods of one hundred and seventeen to one hundred and ninety-one days.

The elimination of the radium was found to be rapid. Approximately 35 per cent was excreted the first day of injection, and a lessening amount thereafter. Only 45 to 50 per cent of the radium was present in the body a week after injection, a large part of this remaining temporarily fixed in the tissues. At death, sixty to seventy-five days after the final injection, 24.6 per cent of the radium had been retained, and all but 1 per cent of this was fairly uniformly distributed throughout the skeleton, paralleling the deposits of calcium. The authors suggested that the rate of the elimination of radium serves as an index to the normal calcium turnover in the bones.

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3. Bade and Küntscher. Récamier and Tribondeau.
4. Sabin and others. Tribondeau and Récamier.

The destructive effect of the radium was described by Thomas and Bruner as follows: "The radium which is present in the bone disintegrates and expends much energy in the form of radiations. Ninety per cent of the energy set free resides in the swift alpha particle." Because of the high kinetic energy of the alpha particle, "it is capable of disrupting molecules, of producing ionization in gases, and of decomposing liquids into their constituent elements." "A rat containing only 15 micrograms of radium in its system absorbs over 2,000,000 alpha particles per second. The 25 ergs of energy so produced exercises a disturbing physiological effect upon the system of the animal. The greater portion of these radiations is concentrated upon the marrow and the immediate tissue. Not only do the bone cells receive the disruptive effect of most of the alpha rays but they also receive the shock of the recoil atoms. The combined effect might cause the destruction of cellular matter, which would be carried away in solution, leaving the bone brittle and fragile."

The most uniform change in the bones was thinning and partial decalcification of the central trabeculae, which resulted in pathologic fracture in at least 3 rats and increased fragility of the bones of all the animals. This was associated with concentration of calcium salts in the parts of the bones nearest the joints. In several animals there was necrosis of bone and also of soft tissue at the costochondral junctions. This was followed by proliferation of fibrous tissue and thickening of the periosteum and endosteum. Similar necrosis and replacement by fibrous tissue was seen in the sternum of 1 animal.

Rabbits that were poisoned and subsequently died from large doses of thorium dioxide showed in the main similar rarefaction of the femoral diaphysis. In addition, a preeminent role of the osteoclasts was suggested by the large numbers massed around the trabeculae (Lambin).

Data from Studies of Man.—Information on the effects of radiation on human bones is incidental to therapeutic irradiation of bones and to poisoning from radioactive substances. Changes resulting from the therapeutic application of radiation are most often seen in the rib, the femur, the mandible and the maxilla after long-continued exposures.

The possibility of stunting bones of children by therapeutic radiation to soft parts over epiphyses must be kept in mind (Walter). Bisgard and Hunt gave 2 illustrative cases. A child 1 year old had radium applied over a nevus on the dorsum of the right index finger. The finger failed to lengthen and at the age of 6, was shorter than the little finger. Roentgenograms showed that all the epiphysial lines of this finger except the proximal phalanx were closed. A child of 5 years had radium and roentgen irradiation of a tumor on the medial aspect of the knee. A varus deformity and shortening of the leg developed. When

this was presented for correction, ten years later, roentgenograms showed that the epiphysial junction on the mesial aspect, which had been nearest the source of radiation had closed but that the junction on the lateral side was open.

Spontaneous fracture resulting from radium poisoning or from therapeutic application of radiation does not usually heal normally; either fibrous union or pseudarthrosis results.[5]

Both absorptive and sclerotic processes, as well as spontaneous fracture, have been observed in the ribs, especially after treatment for carcinoma of the breast.[6]

How often spontaneous fracture of the neck of the femur is a result of injury from radiation during treatment of cancer of the pelvis is not entirely clear. The data are difficult to evaluate, since the normal disadvantages of the neck of the femur as an anatomic unit make it peculiarly liable to fracture from minor trauma. The fact that the incidence of fracture is higher among patients who have had radiation to the pelvis (2.1 per cent of 471 patients—Dalby and co-workers) than in the general population of the same age group is suggestive. In most of the cases the conditions are similar.[7] Fracture usually occurs months after irradiation of the region and is not associated with metastasis. The condition is sometimes bilateral. In cases reported by Dalby and co-workers the initial changes usually developed in the superior portion of the femoral head, close to the neck (Peck). Osteoporosis was often present, but in a case of subcapital fracture of the left hip the heads of both femurs remained dense in spite of atrophy of other bones around the joint (Batt and Hampton). Normal healing is infrequent, but fairly often there is fibrous union. Microscopic studies in 3 cases (Dalby and associates) showed similar changes: extreme obliterative endarteritis of nutrient vessels, "rarefying osteitis" with absorption and partial loss of calcium, and foreign body giant cell reaction about fragments of necrotic bone. Fibrous union occurred in one case, very dense callus in another and pseudarthrosis in the third. In 1 case the neck of the opposite femur showed marked osteoporosis and young fibroblastic proliferation with small islands of cartilage and a slight amount of new bone.

A pure radiation effect is infrequent in the maxilla and the mandible, but destructive lesions of these bones are commonly seen by radiologists as a result of the combined effect of radiation and infection after treatment of intraoral carcinoma. The effect of infection in enhancing damage to bone is well illustrated by the necrosis of the mandible in

5. Flinn. Freid and Goldberg. Jacobsen.

6. Freid and Goldberg. Rose.

7. Bade and Küntscher. Batt and Hampton. Kropp. Philipp.

phosphorus poisoning. Regaud observed certain peculiarities of necrosis of bone following irradiation of carcinoma of the buccal mucosa:

1. Radionecrosis of bone may take place beneath intact skin or mucous membrane.
2. The macroscopic integrity of bone is maintained even after exposure to heavy radiation.
3. Infection or trauma added to radionecrosis produces marked gross disintegration of bone.
4. Sequestration in radionecrosis is much slower than in necrosis due to trauma or infection.
5. Bone killed by radiation is resistant to histolytic solvents.

From these facts Regaud drew the following conclusions: Bone is more vulnerable than surface epithelium. This vulnerability is probably inherent in the supporting substance and not in the bone cells. There is no elective sensitivity of normal adult bone to radiation but rather a diffuse radiosensitivity conditioned by calcification of the fundamental substance. The secondary rays act mainly on the lamellar substance, altering in some way the physical constitution of this substance without producing gross evidence of change as long as the bone remains free from trauma and infection. The macroscopic integrity of heavily irradiated bone is explained by the presence of an amorphous mineral substratum and by the extreme slowness of the normal changes in bone. An alteration in the ground substance is responsible for the greater susceptibility to infection and less susceptibility to histolytic solvents, which is also seen in all collagenous substances affected by radiation.

Ewing made most useful observations of irradiated human bones from the standpoint of histology. He described "productive osteitis" in 3 young adults who had received very heavy roentgen radiation or radium packs, or both, followed in shorter or longer time (three to nine months) by amputation. In case 3 a spontaneous fracture occurred four months after the use of radiation, and eighteen days later, at the time of amputation, there was no trace of callus or of inflammatory reaction. The shaft appeared devitalized and was hard, dense and "polished as ivory," and around it there was new subperiosteal bone, 1 to 3 mm. thick. This could be stripped off with ease, leaving a finely granular bony surface, and suggested an involucrum forming about a sequestrum in the process of absorption.

. . . In the portions of hard bone taken from the shaft away from the tumor, the cell spaces appear to be larger and more irregular, the canaliculi are larger, widened, very irregular, reduced in numbers, and the communications between

adjoining cells are very imperfect. Numerous abnormal spaces appear between the lamellae. The lamellar substance appears very hyaline and brittle. . . . It is admissible to conclude that these changes signify severe degeneration of the bone cells, destruction of many cell processes and closure of many canaliculi and changes of undetermined nature in the lamellar substance.

In the other 2 cases there was an extreme degree of radiation dermatitis together with an impairment of circulation due to vascular changes. In case 1 the bone as a whole did not appear devitalized although some necrosis, decalcification and absorption had occurred, but in case 2 the bone was either necrotic or devitalized and osteoblasts were not seen over a large area. A striking feature of all 3 cases was the brittleness and fragility of the greatly thickened and dense bone. Endosteal bone formation was especially conspicuous.

It has been estimated that poisoning [8] occurs in only 2 per cent of the several thousands of persons exposed in various occupations and in laboratories where radioactive compounds are handled (Ewing).

Regardless of the form in which radioactive material is taken into the system, whether gaseous or solid, the greater part is excreted within a short time, the rapidity of elimination depending partly on the avenue of entry.[9] That which remains is stored in the reticuloendothelial cells. The greatest concentration has been found in the lungs, the spleen and the bones, including the bone matrix (Barker). The rate of elimination varies in different subjects and may continue for many years after exposure (Flinn; Schlundt and Failla). The occurrence of toxic change and the degree of poisoning depend to some extent on the ability of the organism to eliminate radioactive substances and possibly on a certain individual susceptibility. Barker examined the tissues of a person dead of radium poisoning and found the radioactive material hardly sufficient to produce the systemic changes resulting in death.

Pathologic aspects of radium poisoning in man have been reported in considerable detail by Martland, who studied the effect of occupational

8. Rajewsky stated that the "lower limit of the dose which will effect a definite change in the metabolism is about 1.5 x 10^{-5} curie-hours per cubic centimeter. . . . This dose exceeds the normal radium content of the tissues by only about 60 to 90 times and is hence considerably less than the 'tolerance dose' in roentgen or radium therapy." Extremely small amounts of radioactive substance deposited in tissue may produce serious damage. Rajewsky stated that 1 microgram of radium equivalent may produce deadly injury to the organism. To provide this residuum, with the normal rate of elimination, it would be necessary to introduce 100 micrograms orally or 20 micrograms intravenously or by inhalation. The tolerance dose of radium for human tissues is appreciably lower than that of roentgen rays as measured by ionization chambers. This is due chiefly to the great biologic effectiveness of the alpha particles of radium.

9. Barker. Barker and Schlundt. Seil, Viol and Gordon.

exposure to radioactive salts. The workers used a dial paint composed of insoluble sulfates, rendered luminous by small amounts of mesothorium and less radium and radiothorium. The injurious effects were due almost entirely to the disintegration of these substances to form alpha particles. The preponderance of mesothorium in the paint made it particularly toxic, owing to the fact that greater numbers of alpha particles are formed from the disintegration of this substance than from that of either radium or radiothorium (Martland).

At the time of reporting, Martland stated, 18 deaths had been attributed to radium poisoning in the group of exposed persons studied. These deaths occurred three to eight or more years after cessation of exposure. The radioactive substances were confined to the bones, the distribution being fairly uniform throughout the skeleton, with the highest concentration observed in the most heavily calcified portions, as the dense outer cortex. But uniformity of distribution of radium compounds in bone as a usual thing has been questioned by Schlundt and Failla.

The changes in the bone induced by radium are described by Martland as a healing osteitis comparable to the nonsuppurative osteomyelitis of Garré except for the more intense inflammation of the former. Noncellular myxomatous fibrous tissue with many hyperchromatic dividing fibroblasts fills the marrow, which later becomes dense, acellular and ossified. Bone may become nonviable, but necrosis is not usually present unless there is superadded infection, as there commonly is in the jaw (Hoffman, 1925). Unlike phosphorus poisoning, which is the only condition in which an equal degree of necrosis of bone is seen, there is neither involucrum nor healing (Ewing). Osteogenic sarcoma developed in 5 of the 18 persons dying of radium effect reported by Martland. The tumors appeared five to eight years after the contact with radium had ceased. In 2 instances sarcoma was present in two different bones.

Bazy and Coste reported lesions in bones several years after treatment with 4,400 micrograms of thorium dioxide in divided doses. They considered that the lesions resembled those described by Martland in radium poisoning.

Production of Tumors.—Malignant change as a result of exposure to radiant energy has been described somewhat less frequently in bone than in other tissues. Nearly all of the recorded tumors produced in bones of animals have been diagnosed as osteogenic sarcoma, but among the reported tumors 1 was diagnosed as chondrosarcoma (Ludin, 1934), 1 as fibrosarcoma (Daels and Biltris, 1931) and 1 as Ewing's tumor (Schürch and Uehlinger, 1937). Tumors have been described in chickens, rats, mice and a guinea pig as well as in the more commonly used rabbit.

Author	Type of Radiation	Filtration	Time of Radiation	Time from First Exposure to Radiation to Appearance of Tumor or Death	Animal	Type of Tumor
Dacis, F., and Biltris, R., 1931 *	Radium bromide, amount minute but n.s.	.....	.....	2 years and 1 month	Guinea pig	Fibrosarcoma
Schürch, O., and Uehlinger, E., 1931	Radium, 1 mg.	Platinum	20 days	1 year and 6 months	Rabbit	Osteogenic sarcoma
Sabin, F. R.; Doan, C. A., and Forkner, D. E., 1932	Radium chloride in solution, 61.2 micrograms	.....	1 year and 5 months	1 year and 5 months	Rabbit	Osteogenic sarcoma
Schürch, O., and Uehlinger, E., 1935	Radium, 2-5 micrograms, 3 animals	.....	.....	1 year and 9+ months	Rabbit	Osteogenic sarcoma
Ross, J. M., 1936	Radium, 1 mg.	.....	.....	1 year and 11 months	Rabbit	Osteogenic sarcoma
Heliner, H., 1937	Radium, 2,460 milligram hours	.....	Divided doses over 2 years and 3 months	2 years and 3 months	Rabbit	Osteogenic sarcoma
Dacis, F., and Biltris, R., 1937	Radium sulfate, 0.006 mg.	.....	.....	4 years and 10 months	Chicken	Osteogenic sarcoma
Dacis, F., and Biltris, R., 1937	Radium sulfate, 0.003 mg.	.....	.....	4 years and 11 months	Chicken	Osteogenic sarcoma
Sabin, F. R.; Doan, C. A., and Forkner, D. E., 1932.	Mesothorium, 30.6 micrograms	.....	11 months	11 months	Rabbit	Osteogenic sarcoma
Schürch, O., and Uehlinger, E., 1935	Mesothorium, 2-5 micrograms, 2 animals	.....	.....	1 year and 9+ months	Rabbit	Osteogenic sarcoma
Schürch, O., and Uehlinger, E., 1937	Mesothorium, 5 micrograms	.....	.....	3 years	Rabbit	Ewing's tumor
Selbie, F. R., 1938	Colloidal thorium dioxide (thorotrast), 0.2 cc.	.....	.....	1 year and 5 months	Mouse	Osteogenic sarcoma
Lacassagne, A., and Nyka, W. C., 1937	Radon tube, 0.69 millicurie	Glass	.....	1 year and 3 months	Rabbit	Osteogenic sarcoma
Lacassagne, A., and Nyka, W. C., 1937	Radon tube, 0.83 millicurie	Glass	.....	1 year	Rabbit	Osteogenic sarcoma
Lacassagne, A., and Nyka, W. C., 1937	Radon tube, 1.08 millicuries	Glass	.....	1 year and 1 month	Rabbit	Osteogenic sarcoma
Lacassagne, A., and Vinzent, R., 1929	Roentgen rays, 160 kilovolts, 2,000 r in 2 equal exposures, and streptobacillus abscess	.....	6 months	11 months	Rabbit	Osteogenic sarcoma
Lacassagne, A., 1933 *	Roentgen rays, 160 kilovolts, 1,960 r in 3 exposures, and injection of streptobacilli	.....	5 months	5 years and 2 months	Rabbit	Fibrosarcoma
Ludin, M., 1934 †	Roentgen rays, 120 kilovolts, 8,000 r in 40 equal doses	.....	6½ months	6½ months	Rabbit	Chondrosarcoma

* The tumor probably originated from the periosteum, but this is not entirely clear.

† In 1930 Ludin published a short notice of chondrosarcoma arising in the tibia of a guinea pig after a long period of repeated treatments with radiation. Except for the fact that the animal was a guinea pig instead of a rabbit and that details of the experiment were not given, the report was identical with the one described in this table.

As may be seen from the accompanying table of experimentally produced tumors, the factors involved are not easily evaluated. Tumors have followed relatively short as well as long periods of exposure to small and larger amounts of gamma, beta and roentgen rays. Time is an important element, but whether any responsibility for the tumors can be assigned to severe necrosis of bone or chronic inflammation is not clear. Certain of the tumors diagnosed as osteogenic sarcoma reported by Martland in radium dial painters (27 per cent of the deaths resulted from osteogenic sarcoma) arose in the intensely inflamed tissue of radiation osteitis. Several authors have described cancers and benign tumors developing in bones which were the site of tuberculous processes which had been treated by roentgen rays (Selbie). However, bone tumors secondary to inflammation alone are unknown in man, the only recognized predisposing factor being Paget's disease.

Summary.—The reaction of formed bone to radiation is primarily a lowering of vitality which in some instances progresses to necrosis. This may occur without change in the bone's structure or general appearance, the only indication of the injured state being a slight alteration of the osteocytes. Usually injury is indicated by excessive resorption or by overgrowth of bone as if an attempt at repair were being made, and the synchronism of resorption and new bone formation is lost. Atrophy and eburnation of bone are recognized late radiation changes. Irradiated bone, because of the impairment of its vitality, is peculiarly sensitive to infection and trauma and these are in large part responsible for necrosis and sclerosis.

There is little evidence pointing to the reactions of osteocytes, osteoblasts and osteoclasts. On the other hand, the changes in chondroblasts, so carefully studied, demonstrate marked sensitivity of these cells. An equal interest in the gross and the microscopic changes following irradiation of formed bone is much needed.

The matrix of bone is affected, and although the nature of the change is not clearly understood, it may be recognized by certain characteristics, such as (*a*) increased brittleness and (*b*) absence of sequestration.

Adult bone is probably more sensitive to radiant energy than skin or mucous membrane. Although it has received less attention than epiphysial cartilage, there is evidence pointing to like sensitivity.

CARTILAGE

Hyaline cartilage as contrasted with epiphysial cartilage is relatively insensitive to radiation (Dahl) but not to such a degree that its reaction can be neglected when one is considering irradiation of the ear, the nose or the larynx, for example. Perichondritis in these tissues after their

irradiation may be a serious complication, often not fully appreciated at first because of the long period, sometimes several years, before it develops (van Rossem; Schmidt). Changes in the costal cartilages following irradiation of these structures are of no practical importance and are seldom described. Rose, however, mentioned "softening" of cartilage, as well as fibrosis of muscle and of lung and atrophy of bone, some time after heavy radiation had been applied in multiple divided doses for carcinoma of the breast. Many clinical observations of radiation chondritis and perichondritis are available, but the pathologic aspects have been slighted. Indeed, the injury to cartilage has been considered, not altogether erroneously, as due to damage of the blood vessels (Schmidt).

Thies described radiation changes in the ensiform cartilage of the guinea pig. His description is of interest in showing a sequence of cell reactions, various stages of which are familiar to the pathologist. Twenty-four hours after a six hour exposure to 20 mg. of radium bromide the nuclei of the perichondrium and of the superficial cartilage showed various stages of degeneration with accompanying vascular engorgement and infiltration by polymorphonuclears, lymphocytes and a few eosinophils. Six days after irradiation the superficial layer of cartilage appeared broadened and the cells appeared hypertrophied. The perichondrium was markedly thickened and hyperemic. Two weeks later multinucleated cells were present in the superficial cartilage. The matrix was not altered.

Like bone, adult cartilage stands large amounts of radiation without marked gross change so long as it is protected. When it is infected or traumatized, extensive necrosis and sequestration may occur.

TEETH

Experimental studies have emphasized the effect of radiation on developing teeth. Tribondeau and Récamier exposed the anterior lateral aspects of a kitten's face to roentgen rays for ten minutes each six times in a period of two weeks and noted retardation in the development of the teeth, which appeared more marked than that in the development of the bones of the face. Leist and Smith have made similar but more detailed observations.

Leist described changes in the teeth of rats and young dogs exposed to doses for the most part greater than those used for therapy. Eight rats were treated. The radiation was given to the anterior part of the head (120 kilovolts; 28 cm. focal skin distance). The treatment varied: One rat received only 1.5 Holzknecht units; 5 rats, 15 Holzknecht units unfiltered; 2 rats, five and six exposures to 1.5 Holzknecht units each

and later three exposures to 15 Holzknecht units each. Changes were seen only in the growing gnawing teeth. In each case, the odontoblasts were affected and the formation of dentin interrupted, but the enamel and the cement remained unchanged. The injury to the odontoblasts of the germinal layer was more or less severe. Masses of degenerated odontoblasts were found in niches formed in the dentin as a result of the inhibitory effect of the rays. The arrest of growth resulted from the destruction of the odontoblasts.

A similar result was produced by irradiating 3 puppies still with their milk teeth. The jaw alone was irradiated in 3 puppies, in 2 with roentgen rays and in 1 with radium. The roentgen doses varied from 8 to 15 Holzknecht units in a single exposure. One puppy received four treatments, two with filtered and two with unfiltered roentgen rays, two to four weeks apart; the other was given two exposures to unfiltered and one exposure to filtered rays. The third puppy was treated with 100 mg. of radium (in a glass tube enclosed in a silver tube with an outer capsule of platinum 0.3 mm. thick and gutta percha) three times at intervals of four weeks; for one treatment an additional zinc filter was used. The observations were delayed dentition, slowed rate of growth and incomplete development. The odontoblasts were few and much altered in shape, with their nuclei shriveled and disoriented. The pulp showed some early atrophy. Mature teeth were similarly affected but only after 2 to 5 erythema doses. There was also no tendency toward regeneration as in the case of undeveloped teeth.

The amount of radiation determined whether growth was partly or completely stopped, as well as the degree of regeneration. Loss of teeth was in great part due to injury of the peridontal membrane and of the alveolar process itself.

Smith attempted to determine the effect on growing teeth of smaller exposures to radiation. He exposed 5 rats of the same litter, 6 days old, to radiation that was thought "to be equivalent to the individual exposure or repetitions of exposures to which a child might be subjected in securing dental roentgenograms." Exposures were given over a three week period (focal skin distance, 7 inches [7.78 cm.]; 10 milliamperes; spark gap, 3 inches [7.62 cm.]). The time of exposure varied from five to twenty seconds, and from three to six exposures were given weekly. One rat of the same litter served as control. The teeth were examined histologically ten days after the last exposure. Very careful transverse measurements and serial sections of jaws and teeth revealed no structural defects, but the method of examination would not bring out diminution of length. The author explained the difference in his results as compared with those of Leist by the amounts of radiation given in individual doses. Although the cumulative radiation in Smith's

cases equaled some of the individual doses found to be injurious by Leist, the individual doses Smith used were not comparable.

As in the case of phosphorus poisoning, the jaws and the teeth frequently show evidence of injury from radiation insufficient to be manifested by injury elsewhere (Leake).

In minor degrees of radiation injury, sclerosis and atrophy of the alveolus (Leake) have been described, as have fibrosis, "loosening of Sharpey's fibers" (Leist) and loss of vascularity (Tribondeau and Récamier). All these changes indirectly affect the teeth.

The direct effect of radiation on normal teeth has been less clearly understood. In 1926 Flinn stated that "so far as is known, radioactive material has no specific action" on teeth, although dental caries is certainly associated with radium poisoning (Martland). Del Regato stated the problems clearly in his description of changes noted in the teeth of a large number of patients who had been treated for oral or pharyngeal tumors by roentgen rays or radium. He said that it is exceptional for teeth to remain in good condition after exposure to heavy radiation even though they have not been within the field of the direct rays. The evidence of effect is partly subjective, many patients suffering only from hypersensitivity to stimuli during treatment. This sensitivity may disappear at completion of the treatment, but in some cases it reappears several months later, together with obvious dental lesions. The lesions, sluggish in development, as many radiation effects are, progress over a period of years and are generally, although not always, painless. In unusual cases there is complete destruction of teeth within twelve to eighteen months, and this may be very painful. The most common type of lesion is decay of the neck of the teeth, usually on the labial surface of the incisors and canines. The distinctive feature of the decay is its superficial character; it spreads over the surface of the neck before extending deeply and severing the root from the crown, both of which may be sound. In the case of the molars a more general spread of decay over the whole tooth is to be expected.

These lesions are not constant. Del Regato observed that the more severe lesions are seen in patients in whom there has developed intense radioepithelitis of the mucosa of the pharynx after a dose which does not ordinarily produce such a reaction.

Summary.—The direct effect of radiation on mature teeth has not been adequately studied. Such direct injuries as have been described are destruction of odontoblasts, interruption of dentin formation and atrophy of pulp. In growing teeth these injuries inhibit partly or entirely the dental development, with delay or failure of eruption.

Indirect effects, including loss of the teeth, are chiefly produced by injury to the peridontal membrane and of the alveolar process.

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XIII. EFFECTS ON THE SKIN

SHIELDS WARREN, M.D.

The first deleterious effect noted from either roentgen rays or radium was that on the skin and its appendages.[1] By 1902 a series of 167 accidental roentgen ray burns was reported (Codman). The limiting factor in the treatment of many neoplastic processes is the tolerance to radiation of the adjacent or intervening skin. Consequently, more attention has been focused on the effect on this tissue than on any

1. Halkin. Marcuse. Unna.

other in the body. As many reviews of the data are available,[2] only such representative reports as permit giving a general picture of the changes occurring will be selected for consideration here.

The production of visible change in the skin (erythema) has long been used as the biologic standard of therapeutic radiation (Holthusen and Braun). This depends on several variables: the amount of radiation delivered, the amount of radiation absorbed (largely determined by wavelength), the time of exposure (protraction of interval between doses or fractionation of dose greatly decreasing the biologic effectiveness) and the size of the field (the larger field being the more effective because of the amount of back scatter produced). To establish a base for the erythema dose, the following figures from Ellinger are of value: Erythema will be produced in a 6 by 8 cm. field with 400 r of unfiltered soft roentgen radiation, 525 r of medium radiation and 600 r of hard radiation. Hudson, from studies of a number of x-ray apparatus installed in New England, found that with roentgen rays as ordinarily used an erythema may be produced with a dose of approximately 300 r at 100 kilovolts, and with a dose of approximately 700 r at 200 kilovolts. At higher voltages, such as 1,000 kilovolts, no satisfactory method of measuring doses in roentgens has been evolved.

However, with supervoltage the cutaneous reaction ceases to be the limiting factor in radiation therapy. An excellent discussion of the cutaneous reaction to radiation generated at 1,000 kilovolts is given by Stone and Robinson. They expressed the belief that there is no qualitative difference from the reaction obtained at lower voltages and that the assumed variations are due to variations in statement of the dose given. An erythematous reaction similar to that seen following roentgen ray therapy has been produced by a stream of alpha particles (Larkin). Histologically, there is little essential difference in the reactions produced by different types of radiation (Miescher, 1938).

There are two phases of the erythema. The first develops within a few hours to three days after the exposure to radiation and disappears after a day or two. From ten to twenty-eight days later the reddening of the skin again appears and is usually, as it fades, followed by the development of some degree of permanent pigmentation. Pigmentation does not, as a rule, develop after the initial erythema, but only when the more marked, later erythema has occurred. Miescher in 1925 expressed the belief that there are three phases, the maximum histologic changes occurring in the third phase, developing in the sixth week.

The early effects are caused by direct injury of those cells and structures which have been exposed to the radiation. The later effects

2. Desjardins. Flaskamp. McKee. Wetterer.

are brought about by changes in the vascular bed of the corium and in the intercellular tissue and to some extent by changes in the epithelial cells themselves.

The role of the supporting tissues is well shown in the case of the rabbit's ear by transplantation experiments (Ungar and Warren). When a portion of an ear of a rabbit was heavily irradiated (120 millicurie hours of radon in twenty minutes), that epithelium grafted to nonirradiated corium "took" in a number of instances, whereas normal epithelium transplanted to the irradiated corium failed to take.

The early injuries may be shown by chemical as well as histologic change. Thus rabbit skin irradiated with 220 to 1,100 r shows no visible change, but when ashed it shows salts increasing to reach a peak about thirty hours after exposure (Meltzer and Kühtz). There is slight evidence that the cholesterol and phospholipid contents of the stratum corneum are reduced by 900 r, unfiltered, generated at 120 kilovolts (Wile and co-workers). As the acute reaction develops and tissue disintegrates, the skin becomes more acid (Magath), the potassium falls and the calcium rises (Lieber) as does the nonprotein nitrogen (Urbach and Schnitzler).

Several clinical varieties of roentgen or radium injury to the skin are recognized. These are essentially varying manifestations of different intensities of the same fundamental changes.

When the initial erythema has subsided, there is apparently a period of latent injury, which probably represents a period during which the damage to the supporting tissues and to the epithelial cells themselves is gradually developing (Burrows and associates). For ten to twelve days after exposure capillary constriction and dilatation occur to varying degrees but dilatation becomes persistent from about the twelfth to the sixtieth day afterward (Ellinger).

Acute roentgen dermatitis appears as the initial area of erythema begins to fade and to show some slight brownish pigmentation. The skin turns deep red and becomes edematous. Following moderately heavy radiation, this erythema may be accompanied by marked edema and may result in desquamation of the epithelial cells (exudative radiodermitis) to a considerable degree and deposition of fibrin, sometimes accompanied by hemorrhage. This appears at the end of about four weeks of protracted external application of radiation. After about ten days the denuded area has again been covered with epidermis. A lesser degree of reaction is evidenced by scaling of the superficial layers, with bronzing or erythema of the underlying skin (desquamative epidermitis—Cutler and Buschke). These changes correspond to the "epithelitis" of mucous membranes described by Coutard.

Epilation usually occurs.

Sometimes the acute reaction is of the bullous type with large raised blebs containing abundant serum not infrequently becoming secondarily infected. If the radiation has been very heavy, ulcers, sharply defined and punched out, may appear about two months after the exposure. The development of acute ulcers is sometimes precipitated by secondary infection of regions in the irradiated field.

After the erythematous reaction, if the usual therapeutic dose has not been exceeded, there is ordinarily a fair return of the skin to normal, with the exception of some pigmentation due to local increase in melanin. Rarely hemosiderin is present, but it is not an important factor. Ellinger stated that pigmentation induced by roentgen or radium radiation does not migrate to the stratum corneum as does pigmentation caused by light, but rather is restricted to the basal cells of the epidermis and to the chromatophores. When one considers that frequently, following exposure to radiation, the thickness of the stratum corneum may be considerably reduced, the significance of this observation is open to question.

A variable that must be considered is that of susceptibility, individual and specific. Not only do skins of different animal species (Danysz) and of human beings have different thresholds of reaction, but also animals of the same species having different colors will react differently (Rudis-Jicinsky). In general, the heavier the natural pigmentation, the more radiation is required to produce cutaneous injury, as was first pointed out by Rudis-Jicinsky. Danysz showed early that the skin of the guinea pig is more sensitive than that of the rabbit. Even in the same animal parts of the body may vary in susceptibility. Thus, in the human being the exposed skin of extensor surfaces is more resistant than that of protected regions, such as the inner surface of the thigh. The scalp is one of the less sensitive regions (MacKee).

This variation in human susceptibility to injury explains in part the damage done to skin by roentgen ray treatment as administered by operators of beauty shops, through their ignorance (Hazen). It also partly explains why one radiologist may acquire a roentgen ulcer and carcinoma, while another with comparable exposure shows little change in his skin.

The stratum corneum is the portion of the skin most resistant to radiation, largely because its metabolism is relatively inactive and because the keratin is transparent to radiation. On the other hand, the stratum germinativum and the basal layer are relatively sensitive, the hair follicles are very sensitive, and their cells show cessation of mitotic activity and vacuolation before the shaft is lost.

In man epilation may be produced temporarily in about three weeks with about 400 to 500 r at 200 kilovolts and permanently with 700 r or more. The laboratory animals are more resistant than man, as may be seen from the accompanying table.

When animals are temporarily epilated, the hair may grow back white in regions where previously it was pigmented (Hance and Murphy). My associates and I have observed at the periphery of heavily irradiated regions of rabbits' skin a fringe of white hair several millimeters longer than normal.

During the first few days after a dose of radiation producing permanent epilation, the hair follicles show vacuolation, cessation of mitosis and focal necrosis. This is sometimes associated with slight polymorphonuclear and mononuclear infiltration but is generally without leukocytic response. During the first month the arrectores pilorum muscles show some increase in size due to vacuolar degeneration (Wolbach), followed by increasing atrophy, leading ultimately in many instances to complete disappearance.

Doses Producing Permanent Epilation (Adapted from Ellinger [page 32])

Animal	Dose	Type of Radiation and Filtration
Rabbit	2,500 r	200 kv., 2 mm. Cu + 1 mm. Al
Rabbit	2,000 r	100 kv., 2 mm. Cu
Dog	2,400 r	120 kv., 1 mm. Al
Mouse	1,900 r	Hard

The sweat glands are normally as sensitive as are the hair follicles (Flaskamp). The same dose that produces temporary epilation will produce temporary decrease in, or complete disappearance of, the secretion of sweat. Examined microscopically in the first few days, the epithelium shows prominently vacuolar degeneration, which sometimes leads to an unusual degree of distinctness of the so-called myoepithelial cells. About one month after exposure to radiation the basement membrane is doubled or even quadrupled in thickness, the lumens of the glands have virtually disappeared, and the connective tissue adjacent to the coils is increased in amount and hyalinized. Later the sweat glands may disappear entirely.

The sebaceous glands are likewise highly sensitive, and the reduction of their secretion begins within five or six days after exposure (Ellinger). Usually one month after a dose of radiation causing permanent epilation few sebaceous glands will be visible, and those persisting will frequently show some degree of peripheral keratinization, as well as accentuation of the basement membrane. The sebaceous glands tend to persist longer than the hair follicles, but not quite so long as

the coil glands. The dryness and the tendency toward scaling of irradiated skin are largely due to the disturbed function of the coil and sebaceous glands.

The immediate changes in the vascular endothelium are swelling and necrosis. They may be more marked in the endothelium of the capillaries than in that of the lymphatics. These endothelial changes may or may not progress to necrosis and thrombosis (Linser). If endothelial necrosis does not occur, but the damage has been severe, endothelial proliferation with various degrees of occlusion of the lumen may follow. The discussion of the evolution of the vascular lesions has been taken up in chapter VI and will not be repeated here. However, the fundamental importance of these lesions in the development of cutaneous damage must be kept in mind. If the radiation has been sufficiently heavy to produce changes in the vascular bed and in the connective tissue of the corium, this may in turn lead to the development of marked changes in the overlying epithelium. These late changes are seen in their most striking form in the chronic roentgen dermatitis produced by repeated small exposures to radiant energy.

This is most commonly seen on the hands as a result of occupational exposure.[3] The epithelium, which is disturbed in structure as well as in appearance of individual cells, may be hypertrophic or atrophic (Porter). In the hypertrophic form there is marked keratinization of the lesions; also, associated hyperkeratosis of varying size. The epidermis in general is thickened, the hair absent or sparse, the cutaneous folds exaggerated. The nails are brittle, distorted and cracked. This lesion may go on to the development of carcinoma.[4] In the atrophic form the skin is shiny, thin and scaly, revealing beneath it a web of telangiectasis. In such a region a roentgen ulcer may be produced by even minor trauma or infection. It is characterized by sharp edges and a clean base, and is intensely painful. In the reparative epithelium at the margin of such an ulcer, carcinoma may develop.

Roentgen carcinoma may be epidermoid, the more usual type, or it may be of the basal cell type. However, fibrosarcoma may originate rarely from the abnormal fibroblasts of the irradiated corium. Among 135 cases of radiodermatitis in physicians seen at the Mayo Clinic there were 39 in which carcinoma had developed (Leddy and Rigos). Twenty-eight cancers caused by radiation were reported by Witwer and Leucutia, and among these 8 had developed in doctors or dentists.

As far as the connective tissue is concerned, in the early stages of the reaction edema dominates the picture. When radiation effect has been sufficiently severe, there may be deposition of fibrin and necrosis

3. Leddy and Rigos. Saunders and Montgomery.
4. Porter. Wile and others.

of fibroblasts, sometimes accompanied by slight leukocytic infiltration. This occasional necrosis and focal deposition of fibrin may appear even years after the primary radiation injury as a result of abnormalities induced in the fibroblasts when the skin was first irradiated (Wolbach). The collagen fibrils, which at first are only swollen, later tend to coalesce. The hyaline material thus formed is sometimes faintly basophilic and sometimes strikingly acidophilic in staining reaction and is very homogeneous. Among the denser collagen strands there may be some growth of young fibrocytes. Eventually the dense hyalinized collagen makes even more compact a mass than that seen in keloid. If there is appreciable damage of the skin, the basement membrane disappears from below the basal cells about three weeks after exposure to radiation and rarely, if ever, reforms.

Within the first few days after irradiation of skin the elastic fibers are swollen and more readily stained. Later their degeneration may be complete with heavier doses, and their loss is responsible for much of the atrophy and loss of normal contour in the irradiated cutis.

SUMMARY

Erythema is the first clinical evidence of irradiation of the skin. The cutaneous effect depends on alterations produced in the epidermis, the cutaneous appendages, the corium and the vessels. Following erythema, epilation and even ulceration may occur. Pigmentation is usual after doses producing a distinct erythema. Vascular thrombosis or occlusion by endothelial proliferation may damage the blood supply.

In a late radiation reaction, varying degrees of atrophy of the epithelium, hyperkeratosis and scaling may be clearly apparent. The epithelium becomes increasingly thin, with almost complete obliteration of the rete pegs and disappearance of the basement membrane. A considerable degree of distortion of the normal structure may occur, sometimes with the development of abnormal mitotic figures. The cutaneous appendages have partly atrophied or have disappeared. The fibrocytes may show distortion or gigantism. The collagen is dense and hyalinized, without elastic fibers. The vessels are telangiectatic or occluded. Neoplasia is a not infrequent late complication.

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XIV. EFFECTS ON STRIATED MUSCLE

Owing to its high degree of differentiation, striated muscle is resistant to radiation.[1] Even in zones immediately adjacent to complete necrosis due to implantation of unfiltered or lightly filtered radon needles, cross striations are still visible, and the transition from necrotic to apparently normal fibers is abrupt (Lacassagne). In fields where heavy external radiation has produced fibrosis and vascular damage, atrophy and other changes in the muscle fibers may develop as a secondary change. However, some atrophic changes are a direct effect of the radiation (Dobrovolskaia-Zavadskaia).

The experiments of Lacassagne are well planned to show the effect of interstitial radiation. Platinum needles or bare tubes containing

1. Danysz. Englmann. Lacassagne.

radon in amounts of from 5 to 55 millicuries were introduced into the lumbar muscle mass in rabbits. After forty hours there appeared a sharply defined zone of chalky white necrosis, surrounded by a zone of congestion with dilated capillaries and some hemorrhage. This inner zone showed loss of striations, fragmentation of fibers and homogeneity or massing of sarcoplasm. Peripherally, the muscle nuclei appeared normal in spite of these cytoplasmic changes, but farther in the nuclei had been broken in to a dust of chromatin. The amount of radon directly affected the size of the lesions, 5 millicuries producing a zone of necrosis 4 mm. in diameter and 55 millicuries producing a zone 15 mm. in diameter. The necrosis was produced rapidly, and prolonging the time the tubes were left in place changed the picture but little. Moreover, the extent of necrosis was fairly constant regardless of filtration (a radius of about 7 mm. from the tubes).

These experiments were extended by Dobrovolskaia-Zavadskaia, who studied especially cytologic changes in the muscle fibers. She found that the alterations in muscle occurred in more or less concentric zones about a focus of interstitial radiation. (She used from 2 to 14 mg. of radium, left in place from fifteen days to nine and one half months.) The central zone of coagulation necrosis she ascribed to beta radiation; about this she found a zone of atrophy due to gamma radiation. The atrophic changes are not pathognomonic, but resemble those seen in typhoid fever, scarlet fever and some intoxications. The fibers are shrunken, but the nuclei increase in number and tend to cluster together. They persist even when practically all cytoplasm has disappeared. This increase in nuclei (Haendly) and their migration into the sarcoplasm are not unlike what is seen in attempted regeneration of muscle injured by other means. Swelling and rounding of nuclei are not infrequent (Dobrovolskaia-Zavadskaia; Fenn and Latchford). Mitosis is not seen. Karyolysis occurs as a result of treatment with heavy radiation.

In most instances of roentgen therapy, only the atrophic changes appear. Damage to the sarcoplasma, particularly to its contractile elements, is striking. With light radiation there is progressive diminution in volume without striking changes otherwise, whereas, with heavy radiation there is formation of vacuoles, which distort the myofibrils. These vacuoles are usually hydropic but may be fatty (Okada). Sometimes Zenker's degeneration appears. Usually the cross striations are diminished in number or disappear early (Lazarus-Barlow). At the periphery of the zone of atrophy there is frequently a striking contrast between adjacent fibers, some being markedly shrunken, others being virtually unchanged from normal. Occasionally at the periphery of the zone of atrophy there may be even some hypertrophic fibers. With

the disappearance of muscle fibers there is a striking degree of fibrosis (Thies) in which the collagen may become more or less hyalinized. In some of the less damaged fibers there is a striking waviness of the myofibrils that gives them a ribbon-like appearance. In such fibers the longitudinal fibrillation tends to be exaggerated and the cross striations to be somewhat diminished.

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XV. EFFECTS ON THE GERM PLASM AND THE EMBRYO

THE GERM PLASMA

The effects of radiation on the germ plasm were recognized early and have been utilized extensively by experimenters both in botanic and zoologic fields to produce mutations (Muller). Radiation has sometimes caused changes of form of varied severity in those offspring that are viable and has produced effects leading to premature death in others.[1] Since irradiation of the ovaries usually is sufficiently intense to interfere with subsequent development of the ova, this degree of sterility is one of the factors explaining why such radiation is not more often followed by unhealthy or maldeveloped offspring (De Rényi and co-workers).

The studies of Bagg (1929) and Bagg and Little have been of considerable interest. Some of their mice descended from roentgen irradiated parents were followed to the ninth generation (Bagg, 1929). The inheritance of abnormal development was interpreted as mendelian and also recessive to the normal. These defects, occurring among 5,280 experimental animals, did not appear among over 2,000 control animals. The radiation that Bagg gave in these experiments was minimal, being applied for only twelve seconds over the dorsum of the mouse on each of five successive days. The doses would probably not total much more than 100 to 150 r.

As little as 145 r delivered to the ovaries of mice will reduce the fertility of the animals by one-half. The surviving ova either die in

1. Bagg, 1926. Demerec. Flaskamp. Hertwig. Muller, 1933. Perthes.

a short time or are underdeveloped (Martius, 1930). Female mice irradiated with 240 to 280 r become entirely sterile. For the first three to four weeks their matings may prove them fertile. During this period of fertility the size of the litter is reduced and abnormalities of heredity develop in 6 per cent (Snell, 1939; Snell and Ames). Martius (1926) irradiated lightly 5 female mice. Four bore litters, but the litters were small and all the offspring were sterile.

Snell (1933) found that the litters sired by male mice that had been irradiated with 800 r before the onset of sterility were smaller, owing to degeneration of the embryos. He explained this as hypoploidy due to roentgen ray–induced chromosomal aberrations. An interesting avian experiment was carried out by Heim. By giving an erythema dose to the ovaries of hens, he produced cessation of laying for fourteen days. However, when laying was resumed, the chicks born from the eggs were normal. Seven weeks later he gave a full erythema dose, and when laying was resumed, the embryos were either dead or abnormal.

Mutations can be brought about by other means than radiation—for example, by poisoning with certain of the lipoid solvents.

The extensive review of Murphy brought together a considerable mass of experimental information published up to that time (1928). Among the total number of reported offspring, underdevelopment occurred in over 30 per cent, early death in over 25 per cent, gross abnormalities in nearly 30 per cent and weakness in 10 per cent. Some of the offspring showed more than one of these abnormalities.

However, not all results are in accordance with Murphy's. Mice irradiated with 12 per cent, 24 per cent or 36 per cent of an erythema dose produced three generations of normal offspring (Krupshi and Eisenberg). Normal litters were obtained from rabbit does after artificial insemination with spermatozoa irradiated at 200 kilovolts (Asdell and Warren). Doses of radiation heavy enough to effect complete sterilization did not produce any hereditary defects among 3,000 descendants (Dobrovolskaia-Zavadskaia).

Drips and Ford, using 5 to 10 per cent of an erythema dose on rats, found no changes produced in the offspring.

Nurnberger concluded that there was no sound experimental evidence for radiation injury of the germ plasm.

There is a considerable amount of data on man, much of it poorly controlled. One of the larger series is that of Jost, who reported on 141 cases in which radiation was used for various pelvic abnormalities. Among the women treated there were 63 pregnancies; 14 of the women aborted; 41 children were born alive, and all but 1 of these were normal mentally and physically. The child excepted suffered from strabismus and myopia, was underdeveloped and did not speak until it was 3½ years of age.

It has been claimed that temporary sterilization is not injurious to the offspring (Nurnberger; Peller). Pregnancy has been reported in the human being following by three months the intrauterine application of radium (Dawson). Wintz reported that among 500 children of mothers temporarily sterilized none have shown genotypic injuries.

Kaplan reported that of 76 women who had been supposedly sterilized by roentgen irradiation of the ovaries and whose menstruation became reestablished, 44 became pregnant. These produced 47 living children, all of whom were normal. Eight of the 44 aborted. However, it must be remembered that genetic abnormalities, if produced, may not be apparent for several generations.

The reports of Murphy (1928, 1929, 1930) have presented a number of instances of defects produced by radiation injury of the germ plasm. Among 377 radiologists circularized by Hickey and Hall (1927), 37 per cent of the couples were sterile and 4 per cent of 412 children born after employment of a parent in radiation showed abnormalities.

Henshaw, in his article on the biologic significance of the tolerance dose of roentgen rays, and radium, summarizes much of the evidence for genetic injury, concludes the danger is real and feels that the generally accepted tolerance dose of 0.1 r per day may be dangerously large.

THE EMBRYO

While there has been some controversy as to the effect of radiation on the germ plasm, there is general agreement as to the deleterious effect of moderate to heavy radiation on the embryo. Although the data in human cases are not entirely in agreement, the preponderance of evidence establishes for the human being the same general conclusions as can be drawn from animal experiments. Not only may death of the embryo result, but various abnormalities may be produced as well. The nature of these abnormalities varies, some being due to the effect on specific sensitive structures, such as the eye (von Hippel and Pagensteicher) or the epiphysial line, others being due to an effect on the blood vessels with failure of adequate nutrition of a given part, as in some of the deformities of extremities. The claim has been made that indirect irradiation may cause late injury to the fetus (Dautwitz), but the evidence for this is completely unsatisfactory. The early references in this field are of little value because of lack of detail, although it was noted that long irradiation would kill the embryos in utero in guinea pigs (Lengfellner, Maurer), that the effects of radiation were more severe shortly before or soon after birth (Krukenberg) and that abortion was produced in dogs by exposure to roentgen rays (Regaud and co-workers). When pregnant rabbits are heavily irradiated at nearly full term, the young either die at birth or within a short time after birth (Lacassagne and Coutard).

Various reports exist as to the effect in man. Some are equivocal and make one wonder whether a chance abnormality of the fetus has been ascribed to radiation. For example there is the case of a woman irradiated during the third and fourth months of pregnancy, whose child died soon after birth and showed intense general edema (von Spindler).

There is no question, however, that abnormalities are produced in the human as well as in the animal embryo in utero by radiation. They fall into two major groups, one of changes in development of the head, the other of abnormalities of the limbs (Murphy and Goldstein). While those of the limbs have been referred primarily to vascular damage, it is possible that some may be caused by damage to the epiphysial lines. If irradiation of the human pelvis is carried out fairly soon after conception, abortion results. A dose of 600 r at 200 kilovolts is usually sufficient. After the first month (Mayer and coworkers) radiation usually produces fetal injury rather than abortion (Ellinger).

Disturbances of the brain usually occur when radiation is given fairly early in fetal life. Thus, in the rat, the ninth day of pregnancy is the critical period for irradiation of the brain and that for the anlage of the eye is the tenth day (Job and co-workers).

In one series (Murphy) 11 of 24 cases of postconception treatment with radiation terminated in abnormality of the fetus—in the majority of the cases in which this occurred the exposure to radiation took place prior to the fourth month.

One of the largest American series of human embryos irradiated in utero is that of Goldstein and Murphy (1929a). Of 75 children, 38 showed defects or ill health. In 10 the ill health was not due to radiation, but in 28 (37 per cent of the total) it was. Twenty of these 28 showed changes in the central nervous system, and 16 per cent of the group were microcephalic. Goldstein and Murphy (1929b) also reported a case in which microcephalic idiocy followed a cervical treatment of 4400 milligram hours of radium given to the mother for carcinoma during the sixth month of pregnancy.

In 1931 the German national societies interested in heredity and racial hygiene resolved that if a fetus in utero is irradiated the pregnancy should be terminated on eugenic grounds.

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Notes and News

Appointments.—Paul R. Cannon, of University of Chicago, and Robert A. Moore, of Washington University, St. Louis, have been elected to the American Board of Pathology as trustees at large.

Milton G. Bohrod has been appointed pathologist to the Rochester General Hospital, Rochester, N. Y.

Nicholas W. Popoff has resigned as pathologist to the Highland Hospital, Rochester, N. Y.

Awards.—Evarts A. Graham, professor of surgery in Washington University, has received the St. Louis Award given annually by an anonymous donor to the citizen of St. Louis who has made "the most outstanding contribution to the community during the year." The award was made in recognition of Dr. Graham's work on pulmonary and hepatic surgery and his leadership as teacher of students and practitioners.

Howard A. Howe and David Bodian, of the Johns Hopkins University, have been given the Mead Johnson Award by the American Academy of Pediatrics for their work on epidemic poliomyelitis.

The alumni of the New York City Hospital have established the James R. Lisa Award for meritorious research in the laboratories of the hospital.

Yerkes Laboratories of Primate Biology.—The name of Yale Laboratories of Primate Biology, Orange Park, Fla., has been changed to the Yerkes Laboratories of Primate Biology, in honor of Robert M. Yerkes, the founder and director emeritus. The laboratories will be conducted by Yale and Harvard universities. The new director is Karl S. Lashley, and Henry W. Nissen continues as assistant director.

Professorship in Cancer Research.—The Citizen's Aid Society of Minneapolis has given $5,500 a year for five years to the University of Minnesota to support what will be known as the George Chase Christian professorship in cancer research. The first incumbent of the position will be John J. Bittner, at present associate director of the Jackson Memorial Laboratory, Bar Harbor, Maine.

Research in Problems of Sex.—Applications to the Committee for Research in Problems of Sex of the National Research Council, for financial aid during the fiscal year beginning on July 1, in support of work on fundamental problems of sex and reproduction, should be received before April 1. They may be addressed to the chairman, Dr. Robert M. Yerkes, Yale School of Medicine, New Haven, Conn. Although hormonal investigations continue to command the interest and support of the committee, preference, in accordance with current policy, will ordinarily be given to proposals for the investigation of neurologic, psychobiologic and behavioral problems of sex and reproduction.

Book Reviews

The Hemorrhagic Diseases and the Physiology of Hemostasis. Armand J. Quick, Ph.D., M.D., associate professor of pharmacology, Marquette University School of Medicine, Milwaukee. Volume 17. Pp. 340, with 23 figures. Price $5. Springfield, Ill.: Charles C Thomas, Publisher, 1942.

This monograph is an expansion of the author's Beaumont Lectures, delivered in 1941, in which he brought together "the contributions of the laboratory, especially in the field of the coagulation of the blood, and the clinical information on the principal hemorrhagic diseases." The first seven chapters are devoted to the agents concerned in coagulation of the blood and hemostasis: thrombin, prothrombin, fibrinogen, thromboplastin, platelets and anticoagulants. The following hemorrhagic diseases and conditions are described fully in the light of recent developments: thrombopenic purpura; hereditary hemorrhagic diathesis: pseudohemophilia and telangiectasia; afibrinogenemia; hemophilia; hemorrhagic diathesis of avitaminosis K; obstructive jaundice, biliary fistula and damage of the liver. The history, the nature and the actions of heparin and vitamin K are discussed fully. A chapter in the story of vitamin K is summarized as follows: "It will be difficult to find in the annals of medical history a story comparable to the quest of vitamin K. After several groups vied with each other to win the mythical laurels of being the first to synthesize vitamin K, they found at the end of the race, a bottle gathering dust on the laboratory shelf containing a simple chemical, methyl naphthoquinone, which was soon to claim the lion's share of attention. But odd things do occur and no one could predict that a synthetic chemical should show a vitamin activity even greater than the natural product on which mankind and all the higher animals have depended through the ages for the normal coagulation of the blood." There are succinct statements on the classification of hemorrhagic diseases and on hemostasis. In the appendix are described tests, selected on the basis of clinical value, for the study of these diseases. There are good lists of references at the ends of the chapters. The illustrations, mostly charts and curves, are instructive. The author, known for his researches on fundamental problems of blood coagulation, summarizes competently and clearly the present state of knowledge of the hemorrhagic diseases from all points of view. The book will be a landmark in its field.

Books Received

ANNUAL REPORT FOR THE YEAR 1941 OF THE SOUTH AFRICAN INSTITUTE FOR MEDICAL RESEARCH, JOHANNESBURG. E. H. Cluver, M.A., M.D., B.Ch., D.P.H., F.R.S.I., director.

MEDICAL PARASITOLOGY. James T. Culbertson, assistant professor of bacteriology, College of Physicians and Surgeons, Columbia University. Pp. xvi + 285, with 21 plates. Price $4.25. New York: Columbia University Press, 1942.

NEW YORK STATE DEPARTMENT OF HEALTH, EDWARD S. GODFREY JR., M.D., COMMISSIONER. Annual Report of the Division of Laboratories and Research, Augustus B. Wadsworth, M.D., director. Pp. 117. Albany, 1941.

THE SIMS-WOODHEAD MEMORIAL LABORATORY RESEARCH BULLETIN FOR 1939 AND 1940. Papworth, England: Pendragon Press.

CHANGES IN THE KNEE JOINT AT VARIOUS AGES WITH PARTI-REFERENCE TO THE NATURE AND DEVELOPMENT OF DEGENERATIVE JOINT DISEASE. Granville A. Bennett, M.D., associate professor of pathology, Harvard Medical School. Hans Waine, M.D., research fellow in medicine, Harvard Medical School, and graduate assistant in medicine, Massachusetts General Hospital. Walter Bauer, M.D., associate professor in medicine, Harvard Medical School, physician to the Massachusetts General Hospital, and director of the Robert W. Lovett Memorial Foundation for the Study of Crippling Diseases. Pp. 97, with 31 plates. Price $2.50. New York: The Commonwealth Fund, 1942.

SURGICAL PATHOLOGY. William Boyd, M.D., LL.D., M.R.C.P.Ed., F.R.C.P. Lond., Dipl. Psych., F.R.S.C., professor of pathology, University of Toronto. Fifth edition, thoroughly revised. Pp. 843, with 502 illustrations, 16 colored plates. Price $10. Philadelphia: W. B. Saunders Company, 1942.

CONSTITUTION AND DISEASE, APPLIED CONSTITUTIONAL PATHOLOGY. Julius Bauer, M.D., professor of clinical medicine, College of Medical Evangelists, Los Angeles, Calif., and formerly professor of medicine of the University of Vienna. Pp. 208. Price $3.50. New York: Grune & Stratton, 1942.

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